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(54) METHOD FOR DISCRIMINATING TYPES OF ENTEROVIRUS 71 TYPE AND COXSACKIE A GROUP VIRUS 16 TYPE AND DNA PROBE AND DNA FRAGMENT USED THEREFOR

(57)Abstract:
PURPOSE: To enable to discriminate the types of the Enterovirus 71 type (EV 71) and the Coxsackie A group virus 16 type (CA 16) in high accuracy.
CONSTITUTION: The method for discriminating the types of the EV 71 and the CA 16 comprises (i) multiplying a DNA sequence coding for a part of the 5'-nontranslating regions of the EV 71 and the CA 16 and a part of Vp4 and Vp2 proteins having DNA sequences specific to the serum types of the viruses, (ii) determining the DNA sequence of the DNA region coding for the Vp4 protein in the multiplied DNA, (iii) designing DNA sequences complementary to the DNA sequences on the basis of DNA sequences specific to the serum types of the EV 71 and the CA 16 in the DNA sequences, respectively, and subsequently (iv) analyzing an ability for combining the obtained DNA probes with the multiplied DNA obtained by the method (i).

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* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] (i) Enterovirus 71 mold and Coxsackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in each human serum protein type of a part of 5'untranslation region and enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Coxsackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Coxsackie A group virus 16 mold.
[Claim 2] The DNA array which has the DNA field which carries out the code of the ****4 protein of the enterovirus 71 mold under magnification DNA, and a complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach according to claim 1 the DNA array which has the DNA field which carries out the code of the ****4 protein of the Coxsackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.
[Claim 3] The DNA probe which has the magnification DNA including the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41 of the DNA field which carries out the code of the ****4 protein of enterovirus 71 mold, and a complementarity.
[Claim 4] The DNA probe which has the magnification DNA including the DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 of the DNA field which carries out the code of the ****4 protein of Coxsackie A group virus 16 mold, and a complementarity.
[Claim 5] The DNA fragment which is ****4 protein of enterovirus 71 mold and carries out the code of the protein including the amino acid sequence shown by the array number 1 in the amino acid sequence.
[Claim 6] The DNA fragment which is ****4 protein of Coxsackie A group virus 16 mold, and carries out the code of the protein including the amino acid sequence shown by the array number 2 in the amino acid sequence.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] This invention relates to the base sequence in the differentiation approach of the enterovirus 71 mold (this may be written as "EV71" below) belonging to enterovirus, and Coxsackie A group virus 16 mold (this may be written as "CA16" below), the DNA probe used for it, and the gene field which carries out the code of the ****4 protein of enterovirus 71 mold and Coxsackie A group virus 16 mold to a list.
[0002]
[Description of the Prior Art] In order to classify the enterovirus (Enterovirus) belonging to the Picornaviridae (Picornaviridae) into about 70 kinds of human serum protein types and to show a variegated infectious disease, it is difficult to presume the virus which becomes a cause from a clinical manifestation. Therefore, separation identification of a virus is needed for deciding a pathogen. However, a current enterovirus separation method of identification separates a virus using cultivation, and the protection test is still more nearly required for it for identification. And two - four weeks is required for the isolation culture of these viruses. Furthermore, in the protection test which used the neutralization antiserum of a standard stock, the separation stock in which human serum protein type differentiation is impossible appears frequently. This is considered for the gene of enterovirus to vary extremely in a nature at high speed, and production of the antiserum which always neutralizes a fresh separation stock is needed for these solutions.
[0003] High sensitivity and polymerase chain reaction method [Polymerase Chain Reaction which amplifies DNA specifically Law, ; which writes this as the "PCR method" below -- Saiki and others, Science, 230 volumes, p.1350-1354, and 1985 After year reference] is developed, enterovirus The PCR method using a primer complementary to the base sequence of 5'untranslation region, 5' -- in an untranslation region ****4 and ****2 protein To the base sequence of the gene field which carries out a code, a complementarity The primer which it has [Rotbart and H. which are detected by the used PCR method, 5.J.Clinical Microbiology, 28, and 438-442 (1990); Olive and D.M. and 5 J.General Virology, 71, 2141-2147(1990)].
[0004] Furthermore, mold identification of the enterovirus by the stringent reverse solid phase hybridization method using DNA amplified by PCR is reported [clinical [, such as **** Hiroaki,], a virus, 22 volumes, and page 199-207 (1994)]. However, in this experiment, the reactivity of a target gene and a probe changes with differences in an age or an area also in the same human serum protein type, and it is expected to be difficult to select a specific viral strain to a probe. In the disease by enterovirus, since the virus separated from the patient occupies 90% or more by EV71 and CA16, hand, foot and mouth disease can identify the epidemia virus of hand, foot and mouth disease by detecting quickly and simply two sorts of human serum protein types.
[0005]
[Problem(s) to be Solved by the Invention] This invention aims at offer of the approach of carrying out mold differentiation of EV71 and CA16 in a high precision.

[0006]
[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the above-mentioned purpose should be attained, in the base sequence of the gene field which carries out the code of the ****4 protein of EV71 and CA16, this invention persons had a specific base sequence in each human serum protein type, find out that EV71 and CA16 can discriminate in a high precision using this base sequence, and came to complete this invention. That is, this invention provides following 1-6 with the DNA probe and DNA fragment which are used for the mold differentiation approach of the enterovirus 71 mold of a publication, and Coxsackie A group virus 16 mold, and it.
[0007] 1. (i) enterovirus 71 mold and Coxsackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in a part of 5'untranslation region and the human serum protein type of enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Coxsackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Coxsackie A group virus 16 mold.
[0008] 2. DNA Array Which Has DNA Field Which Carries Out Code of the ****4 Protein of Enterovirus 71 Mold under Magnification DNA, and Complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach of one above-mentioned publication that the DNA array which has the gene field which carries out the code of the ****4 protein of the Coxsackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.
3. DNA probe which has magnification DNA including DNA array shown by array number 31, array number 33, array number 35, array number 37, or array number 41 of DNA field which carries out code of ****4 protein of enterovirus 71 mold, and complementarity.
[0009] 4. DNA probe which has magnification DNA including DNA array shown by array number 32, array number 34, array number 36, array number 38, array number 39, or array number 40 of DNA field which carries out code of ****4 protein of Coxsackie A group virus 16 mold, and complementarity. 5. DNA fragment which is ****4 protein of enterovirus 71 mold and carries out code of protein including amino acid sequence shown by array number 1 in the amino acid sequence.
6. DNA fragment which is ****4 protein of Coxsackie A group virus 16 mold, and carries out code of protein including amino acid sequence shown by array number 2 in the amino acid sequence. Hereafter, it explains for details further about this invention.
[0010] Especially in operation of this invention, unless it is directed, the conventional technique in the molecular biology in technical within the limits of the field concerned, microbiology, a recombinant DNA, and immunology is adopted. Such technique is explained in detail into reference. For example, please refer to the following reference. Maniatis, Fitsch, and separate volume (1991); volume for University of Tokyo Institute of Medical Science carcinostatic research sections, and cell technology experiment protocol . Sambrook, MOLECULAR CLONING (1991); A LABORATORY MANUAL (1982); Kevin struhl and others, CURRENT PROTOCOLS IN MORECULAR BIOLOGY, one volume, and two volumes; the volume for Masami Muramatsu, lab manual gene engineering (1990) ; Masami Muramatsu The volume for Hiroto Okayama, a gene engineering handbook, experimental medicine
[0011] 1. Enterovirus 71 Mold And a part of 5'untranslation region of Coxsackie A group virus 16 mold A specific base sequence to the human serum protein type of enterovirus Some ****4 and ****2 proteins which it has a code -- carrying out -- a gene -- a field -- magnification -- enterovirus -- 71 -- a mold -- and -- Coxsackie -- A -- a group -- a virus -- 16 -- a mold -- five -- ' -- an untranslation region -- a part -- enterovirus -- a human serum protein type -- being specific -- a base sequence -- having -- **** -- four -- and -- **** -- two -- protein -- a part -- a code -- carrying out -- a gene -- a field (below this)

Magnification which may be called for short "a gene field including the human serum protein type specific base sequence of enterovirus" can be performed as follows.
[0012] First, the enterovirus isolation culture stock of known [human serum protein type] and the human serum protein type by which subculture is carried out extract RNA from a known enterovirus standard stock etc. with a conventional method, and produces cDNA from this extract RNA using reverse transcriptase. The die length which includes the gene field which carries out the code of 5'untranslation region of enterovirus 71 mold and Coxsackie A group virus 16 mold, and ****4 and ****2 for the oligonucleotide which has a complementarity as a primer in the mold intersection of the upstream of the gene field which includes the human serum protein type specific base sequence of enterovirus in this cDNA, and a down-stream mold intersection amplifies the gene DNA field of about 650 bases.
[0013] the PCR method for which magnification of a gene is usually used -- [-- JP,61-274697,A, JP,62-281,A, 239 Sakai sScience(s), and p.487-491 reference] can perform the detail of this PCR method easily. As long as it uses for coincidence the oligonucleotide which has a complementarity in the mold intersection of the upstream of a gene field including a human serum protein type specific base sequence, and a down-stream mold intersection as an oligonucleotide (this may be called for short a "human serum protein type common primer" below) which can be used as a primer on the occasion of magnification of a gene field including the human serum protein type specific base sequence of enterovirus, you may be what kind of oligonucleotide. It is appropriate to use as a primer the oligonucleotide which was specific to enterovirus, and set the high base sequence of similarity as 5'untranslation region (upstream mold intersection) and ****2 field (down-stream mold intersection) between seeds, and carried out chemosynthesis in them based on the base sequence based on desirable known human serum protein type specific base sequence data.
[0014] As an example of the above-mentioned primer, they are D.M.Olive et al, 71 Journal of General Virology, and a page. 2141-2147 (1990) The oligonucleotide shown by the array number 29 and the array number 30 which are indicated by the oligonucleotide shown by the array number 27 and the array number 28 which are indicated, and the JP,6-311900,A specification which invention-in-this-application persons proposed previously can be mentioned. the inside of these primers -- 5' -- the oligonucleotide shown by the array number 29 as a primer by the side of an end, and 3' -- it is appropriate to use for coincidence the oligonucleotide shown by the array number 28 as a primer by the side of an end.
[0015] Chemosynthesis of the above-mentioned primer can be easily carried out in itself with the solid phase synthesis method using a model 381-ADNA composition machine, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems (Applied Biosystems) company make, etc. It can dissociate with a conventional method, for example, agarose gel electrophoresis, and the above-mentioned magnification DNA can be detected as a DNA band, and, thereby, can check the gene DNA of the enterovirus 71 mold and Coxsackie A group virus 16 mold origin.
[0016] 2. The base sequence of the decision above-mentioned magnification DNA of the base sequence of the gene field which carries out the code of the ****4 protein under magnification DNA is for example, applied biotechnology systems company make and model 373-A. An auto sequencer is used and it is Dye DeoxyTM. It can determine by the terminator method. The base sequence of the gene field which carries out the code of the ****4 protein can be determined by searching for the base sequence [a protein nucleic-acid enzyme, 137 volumes, No. 14, and page 2609-2618 (1992) reference] corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus.
[0017] The array of the gene field which carries out the code of the ****4 protein of EV71 obtained in this way is shown in the array number 3 - the array number 21, and the combination of the amino acid sequence is shown in the array number 1. Moreover, the array of the gene field which carries out the code of the ****4 protein of CA16 is shown in the array number 22 - the array number 26, and the combination of the amino acid sequence is shown in the array number 2. The DNA fragment of this invention which includes the above-mentioned base sequence is the DNA synthesizer usually used only

not only in cDNA compounded from RNA of EV71 and CA16 separation stock, for example, the above-mentioned applied biotechnology systems company make, and model 381-A. It may be compounded using a DNA synthesis machine.
[0018] 3. The base sequence of the DNA probe (this may be called a "EV71 unique probe" for short below) which has a complementarity in the specific base sequence of the design and synthetic enterovirus 71 mold of a DNA probe can be designed by analyzing a specific array to EV71 under array shown by said array number 3 - the array number 21. Similarly, the base sequence of the DNA probe (this may be called a "CA16 unique probe" for short below) which has a complementarity in the specific base sequence of Coxsackie A group virus 16 mold can be designed by analyzing a specific array to CA16 under array shown by said array number 22 - the array number 26.
[0019] As an EV71 unique probe obtained in this way, the oligonucleotide shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 can be mentioned, for example, for example as the oligonucleotide shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41, and a CA16 unique probe. The oligonucleotide shown as an EV71 unique probe by the array number 32, the array number 39, and the array number 40 as the array number 31 and the array number 41, and a CA16 unique probe can be preferably mentioned among the above-mentioned human serum protein type unique probes. The above-mentioned DNA probe is model 381-A, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems company make, in itself. With the solid phase synthesis method using a DNA synthesis machine etc., chemosynthesis can be carried out easily.
[0020] 4. EV71 and CA16 can be judged using a human serum protein type [in which the enterovirus 71 mold and the coxsackie virus 16 mold carried out the mold differentiation above] unique probe as follows. First, viral RNA is extracted from the virus isolation culture stock from the clinical specimen extracted at the time of a medical examination with a conventional method. The gene field which carries out the code of some 5'untranslation region [of enterovirus / a part of], ****4, and ****2 proteins after compounding cDNA using said human serum protein type common primer from this extract RNA is amplified by said PCR method. Detection of Magnification DNA can separate Magnification DNA by agarose electrophoresis, can be made to be able to color it by UV irradiation etc. after dyeing with a suitable stain, for example, the ethidium bromide etc., and can be performed by checking the DNA band.

[0021] For the DNA fragment amplified using said human serum protein type common primer, and detection of said human serum protein type unique DNA probe 71, i.e., EV, mold differentiation of EV71 and CA16 The array shown by the array number 31, the array number 33, the array number 35, the array number 37, and the array number 41 again for example, for detection of CA16 For example, the DNA probe produced by carrying out the indicator of the oligonucleotide which has the array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, and the array number 40 It can be made to be able to hybridize with a conventional method on solid phase, and the class of human serum protein type unique DNA probe can be performed by carrying out detection analysis.
[0022] Although association of DNA to a solid phase top can be performed by carrying out the chemical bond of the approach DNA, for example, the magnification, usually using known in itself with a conventional method to suitable solid phase, for example, a nylon membrane, after denaturalizing The approach (this may be called the "Southern-blotting method" for short below) of neutralizing the above-mentioned agarose gel preferably used in order to check Magnification DNA after denaturalizing, for example, fixing to a nylon membrane after transferring Magnification DNA is mentioned.
[0023] The phosphorylation of 5'end can perform the indicator of a DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make). Moreover, detection of an indicator object can perform the autoradiography of the nylon membrane which performed hybridization with the DNA probe by which the indicator was carried out, and can be performed by investigating the existence of hybridization with each DNA probe.

[0024] Examples, such as a DNA fragment which can be amplified, are shown in drawing 17 using the field which carries out the code of some a part of gene field including above-mentioned human serum protein type specific DNA array of enterovirus used by this invention, i.e., 5'untranslation region of enterovirus, ****4, and ****2 proteins, a human serum protein type common primer, a DNA probe, and this primer. As for the location (base pair number) of the gene field where double digits carry out the code of an array number and the ****4 protein with which a DNA probe combines the figure in ( ), the magnitude of the DNA fragment with which the figure in [ ] is amplified, and bp, a base pair is shown among drawing.
[0025]
[Example] Next, although an example is given and this invention is further explained to a detail, the following example is given only as an aid which acquires recognition concrete about this invention, and the range of this invention is not limited at all by this.
[0026] Example 1 It experimented using the enterovirus separation stock and standard stock with which it dissociated from the patient of (Sequence A) use microorganism following of EV71 and CA16 isolation-culture stock, and the human serum protein type was identified by the protection test using the specific antiserum. The standard stock used here is a virus standard stock by which subculture is carried out in the National Institute of Health.
[0027]
(1) Enterovirus separation stock Stock name (human serum protein type) Separation stage Coxsackie A group virus 16 mold (CA16)
1547/79 1979 4057/81 1981 0216/86 1986 0241/91 1991 Enterovirus 71 mold (EV71)
NAGOYA/70 1970 0108/78 1978 3059/78 1978 3359/83 1983 4132/85 1985 T86236a / 861986 years 1091/89 1989 year 0445 / 90 1990 0004/78 1978 year 1096 / 86 1986 0872/89 1989 year 2587 / 89 1989 2603/89 1989 year 0375 / 90 1990 2136/90 1990 year 2398 / 90 1990 4094 / 90 1990 0419/90 1990
[0028] (2) Standard **** name (human serum protein type) Separation stage Coxsackie A group virus 16 molds (CA16)
Enterovirus 71 molds (EV71)
[0029] (B) From the extract above-mentioned virus liquid of RNA, the SUMAI test R kit (Sumitomo Metal, Ltd. make) extracted RNA, and isopropanol precipitation was performed.
(C) cDNA which originates in each virus using a reverse transcriptase (product made from GIBCO/BRL) by using as mold RNA obtained by the synthetic above-mentioned (B) term of cDNA was compounded.
(D) the oligonucleotide shown by the synthetic array number 28 and the array number 29 of a primer for PCR -- phospho friend DAIDO (Phosphoamidide) -- law -- applied biotechnology systems company make and model 381-A It compounded using the DNA synthesis machine. The above-mentioned oligonucleotide was refined using the OPCTM cartridge, and it was used as a primer of PCR.
[0030] (E) Target's DNA magnification (PCR method)
Reaction mixture is 10mM(s) about final concentration. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 0.55mM MgCl2, 0.05mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 0.1microl, enterovirus cDNA compounded by the aforementioned (C) term 100ng-1microg and Taq polymerase (made in Japanese Roche) 0.125microl (0.625U) -- adding -- total 50microl ** -- what was carried out was prepared. PCR is 95-degree-C 30 seconds, and 55-degree-C reaction condition for 30 seconds, and amplified Target DNA using the amplification system (Amplification System; SHITASU) in 14 cycle.
[0031] Furthermore, they are 10mM(s) about final concentration to the above-mentioned solution. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 2.35mM MgCl2, 0.15mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 1.0microl, Total 50microl which added Taq polymerase 0.25microl (1.25U) The solution was added and it amplified in 95-degree-C 30-second and 55-degree-C 30 seconds, and 72-degree-C 45 seconds and 40 cycle.

[0032] (F) The ethidium bromide was added to agarose gel of 3.0% of checks of the magnification DNA by gel electrophoresis ml 0.5microg /, and electrophoresis of DNA amplified by the above-mentioned (E) term was performed. 254nm ultraviolet rays were irradiated after migration, the coloring reaction of the ethidium bromide detected the DNA band, and the target DNA band of about 650 bases originating in the gene field which carries out the code of a part of 5'untranslation region of enterovirus and some ****4 and ****2 proteins was detected.
[0033] (G) Centricon-100 (Amicon make) is used for the PCR product checked by the decision above (F) of the base sequence of ****4 field, and it is after purification and Dye DeoxyTM. The terminator method Cycle Sequencing It reacts and is model by applied biotechnology systems company 373-A. The base sequence was determined using the DNA sequencer. By searching for the base sequence corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus, the base sequence of the gene field which carries out the code of the ****4 protein was determined. The array which carries out the code of the ****4 protein of CA16 for the array which carries out the code of the ****4 protein of EV71 obtained in this way to the array number 3 - the array number 21 is shown in the array number 22 - the array number 26.
[0034] Example 2 It experimented using the 39 following kinds of human serum protein type enterovirus standard stocks by which subculture is carried out in the mold (differentiation A) use microorganism (1) standard stock National Institute of Health of EV71 and CA16. Each of such enteroviruses is standard stocks with which the human serum protein type is identified by the protection test which used the specific antiserum.
[0035]
A stock name (human serum protein type) A cable address The Coxsackie A group virus 2 molds CA2 ** 4** CA4 ** 6** CA6 ** 7**CA7 ** 9** CA9 ** 10 ** CA10 ** 16 ** CA16 **24** CA24 The Coxsackie B group virus 1 mold CB1 **2** CB2 ** 3** CB3** 4** CB4 ** 5** CB5 **6** CB6 Echovirus 1 mold E1 **3 ** E3 ** 4** E4 ** 5** E5 ** 6** E6 ** 7** E7 ** 9** E9 ** 11 ** E11 ** 12** E12** 13** E13 ** 14** E14** 16**E16 ** 17** E17 **18** E18 ** 19 ** E19 **21** E21** 22** E22 ** 24** E24 ** 25 ** E25 ** 30** E30 Enterovirus 70 mold EV70 ** 71 ** EV71 Poliovirus 1 mold PV1 ** 2 ** PV2 ** 3 ** PV3 [0036] (2) It experimented using the enterovirus separation stock with which it dissociated from the patient of the enterovirus separation stock following, and the human serum protein type was identified by the protection test using the specific antiserum.
[0037]
Stock name Separation stage Coxsackie A group virus 16 mold (CA16)
4057/81 1981 0843/87 1987 0071/88 1988 1045/90 1990 1051/90 1990 0241/90 1990 2500/92 1992 enterovirus 71 mold (EV71)
NAGOYA/70 1970 2105/73 1973 2089/73 1973 2146/73 1973 2203/73 1973 2377/73 1973 2381/73 In 1973 0108/78 1978 3059/78 In 1978 0193/78 1978 0297/78 In 1978 0333/78 1978 0761/82 1982 year FE-27 1982 0272/83 1983 year FE-18 1983 FE-20 1983 4132/85 1985 0866/86 1986 0891/86 In 1986 0948/86 1986 1025/86 In 1986 1027/86 1986 1079/86 1986 1096/86 1986 1163/86 1986 0460/87 1987 0443/88 1988 2587/89 1989 2603 / 891989 years 2136/90 1990 2234/90 1990 2398/90 1990 0419/90 1990 0445/90 1990 0189 / 901990 years 4094/90 1990 FE-45 1990 year FE-46 1990 T91-Y563 1991 1264/92 1992 2326 / 921992 years 2909/93 1993 0089/93 1993 0133/93 1993 0136/93 1993 0146/93 1993 2200/93 1993 year 0154 / 93 1993 0159/93 1993 2122/93 1993 2167/93 1993 2168/93 1993 2173/93 1993 0565/93 1993 0630/93 In 1993 0679/93 1993 0702/93 In 1993 0724/93 1993 0748/93 In 1993 0774/93 1993 0791/93 1993 year FE-57 1993 T93-128 1993 year T93-133 1993 T93-Y998 1993
[0038] (B) As (B) - (F) term of an RNA extract, cDNA composition and magnification by PCR of an enterovirus gene, and its detection example 1 showed, RNA of the above-mentioned enterovirus standard stock and a separation stock was extracted, and magnification was checked in the electrophoresis using agarose gel after amplifying the part corresponding to a part of 5'untranslation region and some ****4 and ****2 proteins. In addition, it is HaeIII of phix174 phage as a marker. The decomposition product was migrated to coincidence.

[0039] (C) The agarose gel used by the (B) term of the Southern blot technique example 2 for the check of Magnification DNA was put in for 30 minutes for 30 minutes with alkali denaturation liquid (0.5M NaOH, 1.5M NaCl) after processing and with neutralization liquid (1 M Tris-HCl, 1.5M NaCl), gel was returned to neutrality, and DNA was denatured. Furthermore, DNA which performed Southern blotting overnight and shifted to the nylon membrane with the conventional method was made to fix by 254nm 1.2x105micro JOULES/cm2 (UV stratalinkerTM; Stratagene make) of ultraviolet rays.
[0040] (D) The base sequence [the array number 26 from the array number 3] of the DNA field which carries out the code of the ****4 protein of CA16 and EV71 shown by a design and the synthetic example 1 (G) term of a human serum protein type specific probe was analyzed, the array number 31 and the array number 41, and the CA16 unique probe were designed like the array number 32, the array number 39, and the array number 40, and the EV71 unique-from these arrays probe compounded according to the example 1 (D) term. The phosphorylation of 5'end performed the indicator of the above-mentioned DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make).
[0041] (E) The membrane produced by the hybridization above-mentioned example 2 (C) term is 1.0M NaCl and 50mM as reaction mixture. Tris-HCl (pH7.5), 0.5%PVP, 0.2% heparin, 1mM It processed at 50 degrees C for 1 hour using EDTA and 2%SDS, and pre hybridization was performed. Hybridization added the [gamma-32P] ATP indicator DNA probe compounded by the (D) term of an example 2 105 cpm/ml to the above-mentioned hybridization solution, and made it react to it 50 degrees C for 4 hours. It washed at 50 degrees C in the 5xSSC-0.1%SDS solution, the membrane was washed twice at 50 degrees C in the 2xSSC-0.1%SDS solution after 5 minutes and 2 times washing for 5 minutes, and the probe combined nonspecific was removed. After drying a nylon membrane, on the lap, after the room temperature performed a package and autoradiography for 15 minutes further for 45 minutes at -80 degrees C, they were developed.
[0042] Formation of the hybrid of the CA16 specific DNA probe (the array number 32, the array number 38, and array number 39) and Magnification DNA which were obtained in this way is shown in drawing 1 - drawing 8 , and the hybridization of an EV71 specific DNA probe (the array number 31 and array number 41) and Magnification DNA is shown in drawing 9 - drawing 16 . The passage clear from drawing, all EV71 separation stocks react to an EV71 specific DNA probe, and all CA16 separation stocks react to a CA16 specific DNA probe, and there is no cross reaction between a human serum protein type specific DNA probe and DNA obtained from the enterovirus standard stock of each human serum protein type, and it became clear that this was specific association. According to the approach of this invention the above-mentioned passage, CA16 and EV71 can discriminate with high precision.
[0043]
[Layout Table]
array number: -- die-length [of one array]: -- mold [of 69 arrays]: -- amino acid topology: -- Ggg of under the information following array of straight chain-like others, and the 3rd amino acid number Ser Or Thr being shown -- Hhh of the 7th amino acid number Ala Or Thr It is shown and is the 9th amino acid number. Iii Arg or -- Xaa -- being shown -- Jjj of the 17th amino acid number Asn or -- Xaa -- being shown -- Kkk of the 31st amino acid number Asn Or Xaa being shown -- Lll of the 34th amino acid number Lys Or Xaa being shown -- Mmm of the 35th amino acid number Asp Or Xaa being shown -- Nnn of the 43rd amino acid number Lys Or Xaa being shown -- Ooo of the 47th amino acid number Lys or Xaa being shown -- Ppp of the 49th amino acid number Asp or Xaa It is shown and is Qqq of the 53rd amino acid number. Phe or Xaa It is shown and is Rrr of the 54th amino acid number. Ala Or Gly Or Xaa It is shown and is Sss of the 55th amino acid number. Asn Or Xaa It is shown.
Array Met Gly Ggg Gln Val Ser Hhh Gln Iii Ser Gly Ser His Glu Asn Ser 5 10 15 Jjj Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr 20 25 30 TyrLll Mmm Ser Tyr Ala Ala Thr Ala Gly Nnn Gln Ser Leu Ooo Gln 35 40 45 Ppp Pro Asp Lys Qqq Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60Ala Ala Pro Leu Lys 65 [0044] array number: -- die-length [of two arrays]: -- mold [of 69 arrays]: -- amino acid topology: -- Aaa of under the information following array of straight chain-like others, and the 9th amino acid number Arg Or Xaa being shown -- Bbb of the 19th amino acid number

Ala Or Xaa being shown -- Ccc of the 21st amino acid number Glu Or Xaa It is shown and is Ddd of the 23rd amino acid number. Ser Or Thr It is shown and is Eee of the 24th amino acid number. Thr Or Xaa It is shown and Fff of the 29th amino acid number is Thr. Or Pro It is shown.
Array Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser 5 10 15 Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr 20 25 30 TyrLys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 Ala Pro ProLeu Lys 65 [0045] array number: -- die-length [of three arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus enterovirus type name: -- the description description of a BrCr array The notation:CDS existence location to express : 1 - 207 description The 1-207th base sequence array ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAC TCA GCC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAA GAC TCG TAT GCT GCC ACT GCT GGA AAG CAA AGT CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys AspIle Phe Thr Glu Met 50 55 60 GCA GCG CCC TTA AAG 207Ala AlaPro Leu Lys 65 [0046] array number: -- die-length [of four arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of NAGOYA/70 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information en TEROUISURU71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAC AAG TTT GGC AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Gly Asn Pro Val Lys AspIle Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG 207Ala Ala Pro Leu Lys 65 [0047] array number: -- die-length [of five arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0108/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCAGGT AAG CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser LeuLys Gln 35 40 45 GAC CCT GAC AAG TTT GCTAAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0048] array number: -- die-length [of six arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71-share name: -- the description description of 3059/73 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT 48 Met Gly Ser Gln Val

Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTT AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln35 40 45 GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro LeuLys 65 [0049] array number: -- die-length [of seven arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 0004/78 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGA TCG CAG GTG TCC ACA CAA CGC TCT GGT TCG CAT GAA AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCC ACT GAA GGT TCC ACT ATA AAC TAC ACC ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAG GAC TCT TAT GCC GCT ACA GCA GGC AAA CAG AGC CTT AAG CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCA CCA CTG AAA 207Ala Ala Pro Leu Lys 65 [0050] array number: -- die-length [of eight arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 3359/83 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAA CAG AGCCTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu LysGln 35 40 45 GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp LysPhe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCG CTGAAG 207Ala Ala Pro Leu Lys65 [0051] array number: -- die-length [of nine arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 4132/85 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAA GTG TCT ACT CAG CGA TCC GGC TCC CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC ANC TAT 96 Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr 20 25 30 TAC ANA GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT AMA CAA 144 Tyr Xaa Xaa Ser Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln 35 40 45 GMC CCT GAT AAG TKT GCT AMT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTA AAA 207Ala Ala Pro Leu Lys65 [0052] array number: -- die-length [of ten arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of a T86236a array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT ACA CAA GTA TCC ACT CAG SGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC

GAG AAT TCT 48 Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCTTAT GCT GCT ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCC GAC AAG TTT GCTAAT CCT GTC AAG GAC ATTTTT ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0053] array number: -- die-length [of 11 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 1096/86 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0054] array number: -- die-length [of 12 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 1091/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0055] array number: -- die-length [of 13 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 share name: -- notation:CDS existence location: showing the description description of 0872/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr ThrThr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TATGCT GCC ACA GCA GGCAAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0056] array number: -- die-length [of 14 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2587/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGC CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala Ala Pro Leu Lys 65 [0057] array number: -- die-length [of 15 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 2603/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GSA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Xaa Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala AlaPro Leu Lys 65 [0058] array number: -- die-length [of 16 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0445/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAC TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0059] array number: -- die-length [of 17 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 0375/90 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0060] array number: -- die-length [of 18 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2136/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT

48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCC ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCT ACA GCAGGC AAA CAG AGC CTCAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala AlaPro Leu Lys 65 [0061] array number: -- die-length [of 19 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2398/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 10 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr IleAsn Tyr 20 30 TAC AAG GAC TCC TAT GCC-GCT-ACA-GCA-GGC AAA CAG AGC CTC AAG CAG 144Tyr Lys Asp Ser Tyr Ala Ala-Thr-Ala-Gly-Lys-Gln-Ser-Leu-Lys-Gln 40 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys 65 [0062] array number: -- die-length [of 20 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71-share name: -- the description description of 4094/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGC TCA CAG GTG TCC ACA CAG CGC TCC GGC TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACC GAG GGC TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAGAGT CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATTTTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAA 207Ala Ala Pro LeuLys 65 [0063] array number: -- die-length [of 21 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0419/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACG CAA CGC TCC GGC TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGT CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTA AAG207Ala Ala Pro Leu Lys 65 [0064] array number: -- die-length [of 22 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of entrovirus coxsackievirus A name: -- the description description of G10 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAA GTC TCA ACC CAA CGA TCG GGT of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCC CAC GAA AAT TCG 48 Met Gly Ser Gln

Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAA GGA TCT ACT ATA AAC TAC ACC ACC ATC AAC TAT 96 Asn Ser Ala Ser Glu Gly Ser ThrIle Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAT GCA TAT GCT GCC AGC GCG GGT CGC CAA GAT ATG TCT CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met SerGln 35 40 45 GAC CCTAAG AAA TTC ACA GAC CCT GTG ATG GAT GTC ATA CAC GAG ATG 192 Asp Pro Lys LysPhe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCT CCC TTG AAA 207Ala Pro Pro Leu Lys65 [0065] array number: -- die-length [of 23 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 1547/79 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTT TCC ACT CAG CGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAT GAG AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCGGGG CGC CAG GAT ATGTCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTT ACCGAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTT AAA 207Ala Pro Pro Leu Lys65 [0066] array number: -- die-length [of 24 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location: showing the description description of 4057/81 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides approach:E which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAG CGG TCT GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAC CCG AAG AAA TTT ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro Leu Lys65 [0067] array number: -- die-length [of 25 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 0216/86 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAC GAA AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GYA TCG glass fiber reinforced gypsum GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC 96 Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr ProIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGACGM CAG GAT ATGTCC CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTC ACCGAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCG CTC AAA 207Ala Pro Pro Leu Lys65 [0068] array number: -- die-length [of 26 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location: showing the description description of 0241/91 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides approach:E which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT

GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAA GAT GCA TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro LeuLys 65 [0069] array number: -- die-length [of 27 arrays]: -- mold [of 16 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CTACTTTGGG TGTCCG which has a complementarity in antigenomic sense RNA 16 [0070] array number: -- die-length [of 28 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the ****2 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in genomic sense RNA. Q of the 6th base number shows T or C during an array, and N of the 18th base number shows A, C, G, or T.
Array GGTAAQTTCC ACCACCANCC 20 [0071] array number: -- die-length [of 29 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CATCTTTGGG TGTCCGTGTT which has a complementarity in antigenomic sense RNA 20 [0072] array number: -- die-length [of 30 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the VP2 protein of information enterovirus besides a synthetic DNA genomic sense Array array TCAGGCAACT TCCACCACCA which has a complementarity in RNA 20 [0073] array number: -- die-length [of 31 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array AGGCTCTGTT TGCCTGCTGT which has a complementarity in antigenomic sense RNA 20 [0074] array number: -- die-length [of 32 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array ATATCCTGGC GCCCCGCACT which has a complementarity in antigenomic sense RNA 20 [0075] array number: -- die-length [of 33 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array TTTGAGGCTC TGTTTGCCTG which has a complementarity in antigenomic sense RNA 20 [0076] array number: -- die-length [of 34 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGACATATCC TGGCGCCCCG which has a complementarity in antigenomic sense RNA 20 [0077] array number: -- die-length [of 35 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGCGCTGCCA TTTCAGTGAA which has a complementarity in antigenomic sense RNA 20 [0078] array number: -- die-length [of 36 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGTGGAGCCA TCTCATGTAT which has a complementarity in antigenomic sense RNA 20 [0079] array number: -- die-length [of 37 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid

chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in antigenomic sense RNA. Q of the 19th base number shows C or T during an array.
Array CATTTCAGTG AAAATRTCQT 20 [0080] array number: -- die-length [of 38 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array CATCTCRTGT ATAACRTCCA which has a complementarity in antigenomic sense RNA 20 [0081] array number: -- die-length [of 39 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array array ATATCTTGAC GCCCAGCGCT which has the complementarity of antigenomic sense RNA 20 [0082] array number: -- die-length [of 40 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 3rd base number shows C or T during an array.
Array ATQTCATGTA TAACRTCCAT 20 [0083] array number: -- die-length [of 41 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 18th base number shows C or T during an array.
Array ATTTCAGTGA AAATRTCQTT 20

[Translation done.]

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TECHNICAL FIELD

[The technical field to which invention belongs] This invention relates to the base sequence in the differentiation approach of the enterovirus 71 mold (this may be written as "EV71" below) belonging to enterovirus, and Coxsackie A group virus 16 mold (this may be written as "CA16" below), the DNA probe used for it, and the gene field which carries out the code of the ****4 protein of enterovirus 71 mold and Coxsackie A group virus 16 mold to a list.

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PRIOR ART

[Description of the Prior Art] In order to classify the enterovirus (Enterovirus) belonging to the Picornaviridae (Picornaviridae) into about 70 kinds of human serum protein types and to show a variegated infectious disease, it is difficult to presume the virus which becomes a cause from a clinical manifestation. Therefore, separation identification of a virus is needed for deciding a pathogen. However, a current enterovirus separation method of identification separates a virus using cultivation, and the protection test is still more nearly required for it for identification. And two - four weeks is required for the isolation culture of these viruses. Furthermore, in the protection test which used the neutralization antiserum of a standard stock, the separation stock in which human serum protein type differentiation is impossible appears frequently. This is considered for the gene of enterovirus to vary extremely in a nature at high speed, and production of the antiserum which always neutralizes a fresh separation stock is needed for these solutions.
[0003] High sensitivity and polymerase chain reaction method [Polymerase Chain Reaction which amplifies DNA specifically Law, ; which writes this as the "PCR method" below -- Saiki and others, Science, 230 volumes, p.1350-1354, and 1985 After year reference] is developed, enterovirus The PCR method using a primer complementary to the base sequence of 5'untranslation region, 5' -- in an untranslation region ****4 and ****2 protein To the base sequence of the gene field which carries out a code, a complementarity The primer which it has [Rotbart and H. which are detected by the used PCR method, 5.J.Clinical Microbiology, 28, and 438-442 (1990); Olive and D.M. and 5 J.General Virology, 71, 2141-2147(1990)].
[0004] Furthermore, mold identification of the enterovirus by the stringent reverse solid phase hybridization method using DNA amplified by PCR is reported [clinical [, such as **** Hiroaki,], a virus, 22 volumes, and page 199-207 (1994)]. However, in this experiment, the reactivity of a target gene and a probe changes with differences in an age or an area also in the same human serum protein type, and it is expected to be difficult to select a specific viral strain to a probe. In the disease by enterovirus, since the virus separated from the patient occupies 90% or more by EV71 and CA16, hand, foot and mouth disease can identify the epidemia virus of hand, foot and mouth disease by detecting quickly and simply two sorts of human serum protein types.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention aims at offer of the approach of carrying out mold differentiation of EV71 and CA16 in a high precision.

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MEANS

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the above-mentioned purpose should be attained, in the base sequence of the gene field which carries out the code of the ****4 protein of EV71 and CA16, this invention persons had a specific base sequence in each human serum protein type, find out that EV71 and CA16 can discriminate in a high precision using this base sequence, and came to complete this invention. That is, this invention provides following 1-6 with the DNA probe and DNA fragment which are used for the mold differentiation approach of the enterovirus 71 mold of a publication, and Coxsackie A group virus 16 mold, and it.
[0007] 1. (i) enterovirus 71 mold and Coxsackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in a part of 5'untranslation region and the human serum protein type of enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Coxsackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Coxsackie A group virus 16 mold.
[0008] 2. DNA Array Which Has DNA Field Which Carries Out Code of the ****4 Protein of Enterovirus 71 Mold under Magnification DNA, and Complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach of one above-mentioned publication that the DNA array which has the gene field which carries out the code of the ****4 protein of the Coxsackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.
3. DNA probe which has magnification DNA including DNA array shown by array number 31, array number 33, array number 35, array number 37, or array number 41 of DNA field which carries out code of ****4 protein of enterovirus 71 mold, and complementarity.
[0009] 4. DNA probe which has magnification DNA including DNA array shown by array number 32, array number 34, array number 36, array number 38, array number 39, or array number 40 of DNA field which carries out code of ****4 protein of Coxsackie A group virus 16 mold, and complementarity.
5. DNA fragment which is ****4 protein of enterovirus 71 mold and carries out code of protein including amino acid sequence shown by array number 1 in the amino acid sequence.
6. DNA fragment which is ****4 protein of Coxsackie A group virus 16 mold, and carries out code of protein including amino acid sequence shown by array number 2 in the amino acid sequence. Hereafter, it explains for details further about this invention.
[0010] Especially in operation of this invention, unless it is directed, the conventional technique in the molecular biology in technical within the limits of the field concerned, microbiology, a recombinant DNA, and immunology is adopted. Such technique is explained in detail into reference. For example,

please refer to the following reference. Maniatis, Fitsch, and separate volume (1991); volume for University of Tokyo Institute of Medical Science carcinostatic research sections, and cell technology experiment protocol . Sambrook, MOLECULAR CLONING (1991); A LABORATORY MANUAL (1982); Kevin struhl and others, CURRENT PROTOCOLS IN MORECULAR BIOLOGY, one volume, and two volumes; the volume for Masami Muramatsu, lab manual gene engineering (1990) ; Masami Muramatsu The volume for Hiroto Okayama, a gene engineering handbook, experimental medicine

[0011] 1. Enterovirus 71 Mold And a part of 5'untranslation region of Coxsackie A group virus 16 mold A specific base sequence to the human serum protein type of enterovirus Some ****4 and ****2 proteins which it has a code -- carrying out -- a gene -- a field -- magnification -- enterovirus -- 71 -- a mold -- and -- Coxsackie -- A -- a group -- a virus -- 16 -- a mold -- five -- ' -- an untranslation region -- a part -- enterovirus -- a human serum protein type -- being specific -- a base sequence -- having -- **** -- four -- and -- **** -- two -- protein -- a part -- a code -- carrying out -- a gene -- a field (below this) Magnification which may be called for short "a gene field including the human serum protein type specific base sequence of enterovirus" can be performed as follows.

[0012] First, the enterovirus isolation culture stock of known [human serum protein type] and the human serum protein type by which subculture is carried out extract RNA from a known enterovirus standard stock etc. with a conventional method, and produces cDNA from this extract RNA using reverse transcriptase. The die length which includes the gene field which carries out the code of 5'untranslation region of enterovirus 71 mold and Coxsackie A group virus 16 mold, and ****4 and ****2 for the oligonucleotide which has a complementarity as a primer in the mold intersection of the upstream of the gene field which includes the human serum protein type specific base sequence of enterovirus in this cDNA, and a down-stream mold intersection amplifies the gene DNA field of about 650 bases.

[0013] the PCR method for which magnification of a gene is usually used -- [-- JP,61-274697,A, JP,62-281,A, 239 Sakai sScience(s), and p.487-491 reference] can perform the detail of this PCR method easily. As long as it uses for coincidence the oligonucleotide which has a complementarity in the mold intersection of the upstream of a gene field including a human serum protein type specific base sequence, and a down-stream mold intersection as an oligonucleotide (this may be called for short a "human serum protein type common primer" below) which can be used as a primer on the occasion of magnification of a gene field including the human serum protein type specific base sequence of enterovirus, you may be what kind of oligonucleotide. It is appropriate to use as a primer the oligonucleotide which was specific to enterovirus, and set the high base sequence of similarity as 5'untranslation region (upstream mold intersection) and ****2 field (down-stream mold intersection) between seeds, and carried out chemosynthesis in them based on the base sequence based on desirable known human serum protein type specific base sequence data.

[0014] As an example of the above-mentioned primer, they are D.M.Olive et al, 71 Journal of General Virology, and a page. 2141-2147 (1990) The oligonucleotide shown by the array number 29 and the array number 30 which are indicated by the oligonucleotide shown by the array number 27 and the array number 28 which are indicated, and the JP,6-311900,A specification which invention-in-this-application persons proposed previously can be mentioned. the inside of these primers -- 5' -- the oligonucleotide shown by the array number 29 as a primer by the side of an end, and 3' -- it is appropriate to use for coincidence the oligonucleotide shown by the array number 28 as a primer by the side of an end.

[0015] Chemosynthesis of the above-mentioned primer can be easily carried out in itself with the solid phase synthesis method using a model 381-ADNA composition machine, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems (Applied Biosystems) company make, etc. It can dissociate with a conventional method, for example, agarose gel electrophoresis, and the above-mentioned magnification DNA can be detected as a DNA band, and, thereby, can check the gene DNA of the enterovirus 71 mold and Coxsackie A group virus 16 mold origin.

[0016] 2. The base sequence of the decision above-mentioned magnification DNA of the base sequence of the gene field which carries out the code of the ****4 protein under magnification DNA is for

example, applied biotechnology systems company make and model 373-A. An auto sequencer is used and it is Dye DeoxyTM. It can determine by the terminator method. The base sequence of the gene field which carries out the code of the ****4 protein can be determined by searching for the base sequence [a protein nucleic-acid enzyme, 137 volumes, No. 14, and page 2609-2618 (1992) reference] corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus.

[0017] The array of the gene field which carries out the code of the ****4 protein of EV71 obtained in this way is shown in the array number 3 - the array number 21, and the combination of the amino acid sequence is shown in the array number 1. Moreover, the array of the gene field which carries out the code of the ****4 protein of CA16 is shown in the array number 22 - the array number 26, and the combination of the amino acid sequence is shown in the array number 2. The DNA fragment of this invention which includes the above-mentioned base sequence is the DNA synthesizer usually used only not only in cDNA compounded from RNA of EV71 and CA16 separation stock, for example, the above-mentioned applied biotechnology systems company make, and model 381-A. It may be compounded using a DNA synthesis machine.

[0018] 3. The base sequence of the DNA probe (this may be called a "EV71 unique probe" for short below) which has a complementarity in the specific base sequence of the design and synthetic enterovirus 71 mold of a DNA probe can be designed by analyzing a specific array to EV71 under array shown by said array number 3 - the array number 21. Similarly, the base sequence of the DNA probe (this may be called a "CA16 unique probe" for short below) which has a complementarity in the specific base sequence of Coxsackie A group virus 16 mold can be designed by analyzing a specific array to CA16 under array shown by said array number 22 - the array number 26.

[0019] As an EV71 unique probe obtained in this way, the oligonucleotide shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 can be mentioned, for example, for example as the oligonucleotide shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41, and a CA16 unique probe. The oligonucleotide shown as an EV71 unique probe by the array number 32, the array number 39, and the array number 40 as the array number 31 and the array number 41, and a CA16 unique probe can be preferably mentioned among the above-mentioned human serum protein type unique probes. The above-mentioned DNA probe is model 381-A, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems company make, in itself. With the solid phase synthesis method using a DNA synthesis machine etc., chemosynthesis can be carried out easily.

[0020] 4. EV71 and CA16 can be judged using a human serum protein type [in which the enterovirus 71 mold and the coxsackie virus 16 mold carried out the mold differentiation above] unique probe as follows. First, viral RNA is extracted from the virus isolation culture stock from the clinical specimen extracted at the time of a medical examination with a conventional method. The gene field which carries out the code of some 5'untranslation region [of enterovirus / a part of], ****4, and ****2 proteins after compounding cDNA using said human serum protein type common primer from this extract RNA is amplified by said PCR method. Detection of Magnification DNA can separate Magnification DNA by agarose electrophoresis, can be made to be able to color it by UV irradiation etc. after dyeing with a suitable stain, for example, the ethidium bromide etc., and can be performed by checking the DNA band.

[0021] For the DNA fragment amplified using said human serum protein type common primer, and detection of said human serum protein type unique DNA probe 71, i.e., EV, mold differentiation of EV71 and CA16 The array shown by the array number 31, the array number 33, the array number 35, the array number 37, and the array number 41 again for example, for detection of CA16 For example, the DNA probe produced by carrying out the indicator of the oligonucleotide which has the array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, and the array number 40 It can be made to be able to hybridize with a conventional method on solid phase, and the class of human serum protein type unique DNA probe can be performed by

carrying out detection analysis.
[0022] Although association of DNA to a solid phase top can be performed by carrying out the chemical bond of the approach DNA, for example, the magnification, usually using known in itself with a conventional method to suitable solid phase, for example, a nylon membrane, after denaturalizing The approach (this may be called the "Southern-blotting method" for short below) of neutralizing the above-mentioned agarose gel preferably used in order to check Magnification DNA after denaturalizing, for example, fixing to a nylon membrane after transferring Magnification DNA is mentioned.
[0023] The phosphorylation of 5'end can perform the indicator of a DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make). Moreover, detection of an indicator object can perform the autoradiography of the nylon membrane which performed hybridization with the DNA probe by which the indicator was carried out, and can be performed by investigating the existence of hybridization with each DNA probe.
[0024] Examples, such as a DNA fragment which can be amplified, are shown in drawing 17 using the field which carries out the code of some a part of gene field including above-mentioned human serum protein type specific DNA array of enterovirus used by this invention, i.e., 5'untranslation region of enterovirus, ****4, and ****2 proteins, a human serum protein type common primer, a DNA probe, and this primer. As for the location (base pair number) of the gene field where double digits carry out the code of an array number and the ****4 protein with which a DNA probe combines the figure in ( ), the magnitude of the DNA fragment with which the figure in [ ] is amplified, and bp, a base pair is shown among drawing.

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

EXAMPLE

[Example] Next, although an example is given and this invention is further explained to a detail, the following example is given only as an aid which acquires recognition concrete about this invention, and the range of this invention is not limited at all by this.
[0026] Example 1 It experimented using the enterovirus separation stock and standard stock with which it dissociated from the patient of (Sequence A) use microorganism following of EV71 and CA16 isolation-culture stock, and the human serum protein type was identified by the protection test using the specific antiserum. The standard stock used here is a virus standard stock by which subculture is carried out in the National Institute of Health.
[0027]
(1) Enterovirus separation stock Stock name (human serum protein type) Separation stage Coxsackie A group virus 16 mold (CA16)
1547/79 1979 4057/81 1981 0216/86 1986 0241/91 1991 Enterovirus 71 mold (EV71)
NAGOYA/70 1970 0108/78 1978 3059/78 1978 3359/83 1983 4132/85 1985 T86236a / 861986 years 1091/89 1989 year 0445 / 90 1990 0004/78 1978 year 1096 / 86 1986 0872/89 1989 year 2587 / 89 1989 2603/89 1989 year 0375 / 90 1990 2136/90 1990 year 2398 / 90 1990 4094 / 90 1990 0419/90 1990
[0028] (2) Standard **** name (human serum protein type) Separation stage Coxsackie A group virus 16 molds (CA16)
Enterovirus 71 molds (EV71)
[0029] (B) From the extract above-mentioned virus liquid of RNA, the SUMAI test R kit (Sumitomo Metal, Ltd. make) extracted RNA, and isopropanol precipitation was performed.
(C) cDNA which originates in each virus using a reverse transcriptase (product made from GIBCO/BRL) by using as mold RNA obtained by the synthetic above-mentioned (B) term of cDNA was compounded.
(D) the oligonucleotide shown by the synthetic array number 28 and the array number 29 of a primer for PCR -- phospho friend DAIDO (Phosphoamidide) -- law -- applied biotechnology systems company make and model 381-A It compounded using the DNA synthesis machine. The above-mentioned oligonucleotide was refined using the OPCTM cartridge, and it was used as a primer of PCR.
[0030] (E) Target's DNA magnification (PCR method)
Reaction mixture is 10mM(s) about final concentration. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 0.55mM MgCl2, 0.05mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 0.1microl, enterovirus cDNA compounded by the aforementioned (C) term 100ng-1microg and Taq polymerase (made in Japanese Roche) 0.125microl (0.625U) -- adding -- total 50microl ** -- what was carried out was prepared. PCR is 95-degree-C 30 seconds, and 55-degree-C reaction condition for 30 seconds, and amplified Target DNA using the amplification system (Amplification System; SHITASU) in 14 cycle.
[0031] Furthermore, they are 10mM(s) about final concentration to the above-mentioned solution. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 2.35mM MgCl2, 0.15mM(s) In dNTPs and the solution

made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 1.0microl, Total 50microl which added Taq polymerase 0.25microl (1.25U) The solution was added and it amplified in 95-degree-C 30-second and 55-degree-C 30 seconds, and 72-degree-C 45 seconds and 40 cycle.
[0032] (F) The ethidium bromide was added to agarose gel of 3.0% of checks of the magnification DNA by gel electrophoresis ml 0.5microg /, and electrophoresis of DNA amplified by the above-mentioned (E) term was performed. 254nm ultraviolet rays were irradiated after migration, the coloring reaction of the ethidium bromide detected the DNA band, and the target DNA band of about 650 bases originating in the gene field which carries out the code of a part of 5'untranslation region of enterovirus and some ****4 and ****2 proteins was detected.
[0033] (G) Centricon-100 (Amicon make) is used for the PCR product checked by the decision above (F) of the base sequence of ****4 field, and it is after purification and Dye DeoxyTM. The terminator method Cycle Sequencing It reacts and is model by applied biotechnology systems company 373-A. The base sequence was determined using the DNA sequencer. By searching for the base sequence corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus, the base sequence of the gene field which carries out the code of the ****4 protein was determined. The array which carries out the code of the ****4 protein of CA16 for the array which carries out the code of the ****4 protein of EV71 obtained in this way to the array number 3 - the array number 21 is shown in the array number 22 - the array number 26.
[0034] Example 2 It experimented using the 39 following kinds of human serum protein type enterovirus standard stocks by which subculture is carried out in the mold (differentiation A) use microorganism (1) standard stock National Institute of Health of EV71 and CA16. Each of such enteroviruses is standard stocks with which the human serum protein type is identified by the protection test which used the specific antiserum.
[0035]
A stock name (human serum protein type) A cable address The Coxsackie A group virus 2 molds CA2 ** 4** CA4 ** 6** CA6 ** 7** CA7 ** 9** CA9** 10 ** CA10 ** 16 ** CA16 **24** CA24 The Coxsackie B group virus 1 mold CB1 **2** CB2 ** 3** CB3** 4** CB4 ** 5** CB5** 6** CB6 Echovirus 1 mold E1 ** 3** E3 **4** E4 ** 5** E5 ** 6** E6 ** 7** E7 ** 9** E9 ** 11 ** E11 ** 12** E12** 13** E13** 14** E14** 16** E16 ** 17** E17 ** 18** E18 ** 19 ** E19 ** 21** E21 ** 22** E22 ** 24 ** E24 ** 25** E25 ** 30 ** E30 Enterovirus 70 molds EV70 ** 71**EV71 Poliovirus 1 mold PV1 ** 2 ** PV2 ** 3 ** PV3 [0036] (2) It experimented using the enterovirus separation stock with which it dissociated from the patient of the enterovirus separation stock following, and the human serum protein type was identified by the protection test using the specific antiserum.
[0037]
Stock name Separation stage Coxsackie A group virus 16 mold (CA16)
4057/81 1981 0843/87 1987 0071/88 1988 1045/90 1990 1051/90 1990 0241/90 1990 2500/92 1992 enterovirus 71 mold (EV71)
NAGOYA/70 1970 2105/73 1973 2089/73 1973 2146/73 1973 2203/73 1973 2377/73 1973 2381/73 In 1973 0108/78 1978 3059/78 In 1978 0193/78 1978 0297/78 In 1978 0333/78 1978 0761/82 1982 year FE-27 1982 0272/83 1983 year FE-18 1983 FE-20 1983 4132/85 1985 0866/86 1986 0891/86 In 1986 0948/86 1986 1025/86 In 1986 1027/86 1986 1079/86 1986 1096/86 1986 1163/86 1986 0460/87 1987 0443/88 1988 2587/89 1989 2603 / 891989 years 2136/90 1990 2234/90 1990 2398/90 1990 0419/90 1990 0445/90 1990 0189 / 901990 years 4094/90 1990 FE-45 1990 year FE-46 1990 T91-Y563 1991 1264/92 1992 2326 / 921992 years 2909/93 1993 0089/93 1993 0133/93 1993 0136/93 1993 0146/93 1993 2200/93 1993 year 0154 / 93 1993 0159/93 1993 2122/93 1993 2167/93 1993 2168/93 1993 2173/93 1993 0565/93 1993 0630/93 In 1993 0679/93 1993 0702/93 In 1993 0724/93 1993 0748/93 In 1993 0774/93 1993 0791/93 1993 year FE-57 1993 T93-128 1993 year T93-133 1993 T93-Y998 1993
[0038] (B) As (B) - (F) term of an RNA extract, cDNA composition and magnification by PCR of an enterovirus gene, and its detection example 1 showed, RNA of the above-mentioned enterovirus

standard stock and a separation stock was extracted, and magnification was checked in the electrophoresis using agarose gel after amplifying the part corresponding to a part of 5'untranslation region and some ****4 and ****2 proteins. In addition, it is HaeIII of phix174 phage as a marker. The decomposition product was migrated to coincidence.

[0039] (C) The agarose gel used by the (B) term of the Southern blot technique example 2 for the check of Magnification DNA was put in for 30 minutes for 30 minutes with alkali denaturation liquid (0.5M NaOH, 1.5M NaCl) after processing and with neutralization liquid (1 M Tris-HCl, 1.5M NaCl), gel was returned to neutrality, and DNA was denatured. Furthermore, DNA which performed Southern blotting overnight and shifted to the nylon membrane with the conventional method was made to fix by 254nm 1.2x105micro JOULES/cm2 (UV stratalinkerTM; Stratagene make) of ultraviolet rays.

[0040] (D) The base sequence [the array number 26 from the array number 3] of the DNA field which carries out the code of the ****4 protein of CA16 and EV71 shown by a design and the synthetic example 1 (G) term of a human serum protein type specific probe was analyzed, the array number 31 and the array number 41, and the CA16 unique probe were designed like the array number 32, the array number 39, and the array number 40, and the EV71 unique-from these arrays probe compounded according to the example 1 (D) term. The phosphorylation of 5'end performed the indicator of the above-mentioned DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make).

[0041] (E) The membrane produced by the hybridization above-mentioned example 2 (C) term is 1.0M NaCl and 50mM as reaction mixture. Tris-HCl (pH7.5), 0.5%PVP, 0.2% heparin, 1mM It processed at 50 degrees C for 1 hour using EDTA and 2%SDS, and pre hybridization was performed. Hybridization added the [gamma-32P] ATP indicator DNA probe compounded by the (D) term of an example 2 105 cpm/ml to the above-mentioned hybridization solution, and made it react to it 50 degrees C for 4 hours. It washed at 50 degrees C in the 5xSSC-0.1%SDS solution, the membrane was washed twice at 50 degrees C in the 2xSSC-0.1%SDS solution after 5 minutes and 2 times washing for 5 minutes, and the probe combined nonspecific was removed. After drying a nylon membrane, on the lap, after the room temperature performed a package and autoradiography for 15 minutes further for 45 minutes at -80 degrees C, they were developed.

[0042] Formation of the hybrid of the CA16 specific DNA probe (the array number 32, the array number 38, and array number 39) and Magnification DNA which were obtained in this way is shown in drawing 1 - drawing 8 , and the hybridization of an EV71 specific DNA probe (the array number 31 and array number 41) and Magnification DNA is shown in drawing 9 - drawing 16 . The passage clear from drawing, all EV71 separation stocks react to an EV71 specific DNA probe, and all CA16 separation stocks react to a CA16 specific DNA probe, and there is no cross reaction between a human serum protein type specific DNA probe and DNA obtained from the enterovirus standard stock of each human serum protein type, and it became clear that this was specific association. According to the approach of this invention the above-mentioned passage, CA16 and EV71 can discriminate with high precision.

[0043]

[Layout Table]

array number: -- die-length [of one array]: -- mold [of 69 arrays]: -- amino acid topology: -- Ggg of under the information following array of straight chain-like others, and the 3rd amino acid number Ser Or Thr being shown -- Hhh of the 7th amino acid number Ala Or Thr It is shown and is the 9th amino acid number. Iii Arg or -- Xaa -- being shown -- Jjj of the 17th amino acid number Asn or -- Xaa -- being shown -- Kkk of the 31st amino acid number Asn Or Xaa being shown -- Lll of the 34th amino acid number Lys Or Xaa being shown -- Mmm of the 35th amino acid number Asp Or Xaa being shown -- Nnn of the 43rd amino acid number Lys Or Xaa being shown -- Ooo of the 47th amino acid number Lys or Xaa being shown -- Ppp of the 49th amino acid number Asp or Xaa It is shown and is Qqq of the 53rd amino acid number. Phe or Xaa It is shown and is Rrr of the 54th amino acid number. Ala Or Gly Or Xaa It is shown and is Sss of the 55th amino acid number. Asn Or Xaa It is shown.

Array Met Gly Ggg Gln Val Ser Hhh Gln Iii Ser Gly Ser His Glu Asn Ser 5 10 15 Jjj Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr 20 25 30 TyrLll Mmm Ser Tyr Ala Ala Thr Ala Gly Nnn

Gln Ser Leu Ooo Gln 35 40 45 Ppp Pro Asp Lys Qqq Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60Ala Ala Pro Leu Lys 65 [0044] array number: -- die-length [of two arrays]: -- mold [of 69 arrays]: -- amino acid topology: -- Aaa of under the information following array of straight chain-like others, and the 9th amino acid number Arg Or Xaa being shown -- Bbb of the 19th amino acid number Ala Or Xaa being shown --`Ccc of the 21st amino acid number Glu Or Xaa It is shown and is Ddd of the 23rd amino acid number. Ser Or Thr It is shown and is Eee of the 24th amino acid number. Thr Or Xaa It is shown and Fff of the 29th amino acid number is Thr. Or Pro It is shown.
Array Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser 5 10 15 Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr 20 25 30 TyrLys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 Ala Pro ProLeu Lys 65 [0045] array number: -- die-length [of three arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus enterovirus type name: -- the description description of a BrCr array The notation:CDS existence location to express : 1 - 207 description The 1-207th base sequence array ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAC TCA GCC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAA GAC TCG TAT GCT GCC ACT GCT GGA AAG CAA AGT CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys AspIle Phe Thr Glu Met 50 55 60 GCA GCG CCC TTA AAG 207Ala AlaPro Leu Lys 65 [0046] array number: -- die-length [of four arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of NAGOYA/70 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information en TEROUISURU71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln-Ser-Leu-Lys-Gln 35 40 45 GAC CCT GAC AAG TTT GGC-AAT-CCT-GTC-AAG GAC ATT TTC ACT GAA ATG 192Asp Pro Asp Lys Phe Gly Asn-Pro-Val-Lys-Asp-Ile-Phe-Thr-Glu-Met 50 55 60 GCG GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0047] array number: -- die-length [of five arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0108/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCAGGT AAG CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser LeuLys Gln 35 40 45 GAC CCT GAC AAG TTT GCTAAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0048] array number: -- die-length [of six arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type

71-share name: -- the description description of 3059/73 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTT AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln35 40 45 GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro LeuLys 65 [0049] array number: -- die-length [of seven arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 Stock name : the description description of 0004/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGA TCG CAG GTG TCC ACA CAA CGC TCT GGT TCG CAT GAA AAT TCT of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCC ACT GAA GGT TCC ACT ATA AAC TAC ACC ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAG GAC TCT TAT GCC GCT ACA GCA GGC AAA CAG AGC CTTAAG CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG 192 Asp ProAsp Lys Phe Ala Asn ProVal Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCA CCA CTG AAA 207Ala Ala Pro Leu Lys 65 [0050] array number: -- die-length [of eight arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 3359/83 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAA CAG AGCCTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu LysGln 35 40 45 GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp LysPhe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCG CTGAAG 207Ala Ala Pro Leu Lys65 [0051] array number: -- die-length [of nine arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 4132/85 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAA GTG TCT ACT CAG CGA TCC GGC TCC CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC ANC TAT 96 Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr 20 25 30 TAC ANA GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT AMA CAA 144 Tyr Xaa Xaa Ser Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln 35 40 45 GMC CCT GAT AAG TKT GCT AMT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTA AAA 207Ala Ala Pro Leu Lys65 [0052] array number: -- die-length [of ten arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -

- the description description of a T86236a array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT ACA CAA GTA TCC ACT CAG SGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCTTAT GCT GCT ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCC GAC AAG TTT GCTAAT CCT GTC AAG GAC ATTTTT ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0053] array number: -- die-length [of 11 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 1096/86 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0054] array number: -- die-length [of 12 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 1091/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0055] array number: -- die-length [of 13 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 share name: -- notation:CDS existence location: showing the description description of 0872/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr ThrThr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TATGCT GCC ACA GCA GGCAAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0056] array number: -- die-length [of 14 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description

description of 2587/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGC CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala Ala Pro Leu Lys 65 [0057] array number: -- die-length [of 15 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 2603/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GSA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Xaa Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0058] array number: -- die-length [of 16 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0445/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAC TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0059] array number: -- die-length [of 17 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 0375/90 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0060] array number: -- die-length [of 18 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2136/90 array The

notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCC ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCT ACA GCAGGC AAA CAG AGC CTCAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala AlaPro Leu Lys 65 [0061] array number: -- die-length [of 19 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 2398/90 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 10 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 AsnSer Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 30 TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAG AGC CTC AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 40 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys 65 [0062] array number: -- die-length [of 20 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71-share name: -- the description description of 4094/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGC TCA CAG GTG TCC ACA CAG CGC TCC GGC TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACC GAG GGC TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAGAGT CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATTTTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAA 207Ala Ala Pro LeuLys 65 [0063] array number: -- die-length [of 21 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0419/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACG CAA CGC TCC GGC TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGT CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln-Ser-Leu-Lys-Gln 35 40 45 GAT CCA GAC AAG TTT GCA-AAT-CCT-GTC-AAA GAT ATT TTC ACT GAA ATG 192Asp Pro Asp Lys Phe Ala Asn-Pro-Val-Lys-Asp-Ile-Phe-Thr-Glu-Met 50 55 60 GCT GCG CCA CTA AAG 207Ala Ala Pro Leu Lys 65 [0064] array number: -- die-length [of 22 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of entrovirus coxsackievirus A name: -- the description description of G10 array The notation:CDS existence location

to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAA GTC TCA ACC CAA CGA TCG GGT of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCC CAC GAA AAT TCG 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAA GGA TCT ACT ATA AAC TAC ACC ACC ATC AAC TAT 96 Asn Ser Ala Ser Glu Gly Ser ThrIle Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAT GCA TAT GCT GCC AGC GCG GGT CGC CAA GAT ATG TCT CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met SerGln 35 40 45 GAC CCTAAG AAA TTC ACA GAC CCT GTG ATG GAT GTC ATA CAC GAG ATG 192 Asp Pro Lys LysPhe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCT CCC TTG AAA 207Ala Pro Pro Leu Lys65 [0065] array number: -- die-length [of 23 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 1547/79 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTT TCC ACT CAG CGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAT GAG AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCGGGG CGC CAG GAT ATGTCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTT ACCGAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTT AAA 207Ala Pro Pro Leu Lys65 [0066] array number: -- die-length [of 24 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- coxsackievirus A16 Stock name : the description description of 4057/81 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTC TCC ACT CAG CGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser ThrIle Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met SerGln 35 40 45 GAC CCGAAG AAA TTT ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys LysPhe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro Leu Lys65 [0067] array number: -- die-length [of 25 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 0216/86 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAC GAA AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GYA TCG glass fiber reinforced gypsum GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC 96 Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr ProIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGACGM CAG GAT ATGTCC CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTC ACCGAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCG CTC AAA 207Ala Pro Pro Leu Lys65 [0068] array number: -- die-length [of 26 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location: showing the

description description of 0241/91 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides approach:E which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAA GAT GCA TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro Leu Lys65 [0069] array number: -- die-length [of 27 arrays]: -- mold [of 16 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CTACTTTGGG TGTCCG which has a complementarity in antigenomic sense RNA 16 [0070] array number: -- die-length [of 28 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the ****2 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in genomic sense RNA. Q of the 6th base number shows T or C during an array, and N of the 18th base number shows A, C, G, or T.
Array GGTAAQTTCC ACCACCANCC 20 [0071] array number: -- die-length [of 29 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CATCTTTGGG TGTCCGTGTT which has a complementarity in antigenomic sense RNA 20 [0072] array number: -- die-length [of 30 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the VP2 protein of information enterovirus besides a synthetic DNA genomic sense Array array TCAGGCAACT TCCACCACCA which has a complementarity in RNA 20 [0073] array number: -- die-length [of 31 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array AGGCTCTGTT TGCCTGCTGT which has a complementarity in antigenomic sense RNA 20 [0074] array number: -- die-length [of 32 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array ATATCCTGGC GCCCCGCACT which has a complementarity in antigenomic sense RNA 20 [0075] array number: -- die-length [of 33 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array TTTGAGGCTC TGTTTGCCTG which has a complementarity in antigenomic sense RNA 20 [0076] array number: -- die-length [of 34 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGACATATCC TGGCGCCCCG which has a complementarity in antigenomic sense RNA 20 [0077] array number: -- die-length [of 35 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGCGCTGCCA TTTCAGTGAA which has a complementarity in antigenomic sense RNA 20 [0078] array number: -- die-length [of 36 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene which

carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGTGGAGCCA TCTCATGTAT which has a complementarity in antigenomic sense RNA 20 [0079] array number: -- die-length [of 37 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in antigenomic sense RNA. Q of the 19th base number shows C or T during an array.
Array CATTTCAGTG AAAATRTCQT 20 [0080] array number: -- die-length [of 38 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array CATCTCRTGT ATAACRTCCA which has a complementarity in antigenomic sense RNA 20 [0081] array number: -- die-length [of 39 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array array ATATCTTGAC GCCCAGCGCT which has the complementarity of antigenomic sense RNA 20 [0082] array number: -- die-length [of 40 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 3rd base number shows C or T during an array.
Array ATQTCATGTA TAACRTCCAT 20 [0083] array number: -- die-length [of 41 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 18th base number shows C or T during an array.
Array ATTTCAGTGA AAATRTCQTT 20

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 2] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 3] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 4] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 5] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 6] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 7] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 8] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.
[Drawing 9] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 10] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 11] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 12] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 13] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 14] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 15] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 16] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.
[Drawing 17] It is drawing showing the joint location of a CA16 unique DNA probe and an EV71 unique DNA probe in the location of the magnification gene field of enterovirus, and the magnitude list of Magnification DNA. The location in the gene field where the figure in an array number and ( ) carries out the code of the ****4 protein in two digits, and the figure in [ ] show the magnitude (bp) of a DNA fragment among drawing.

[Translation done.]

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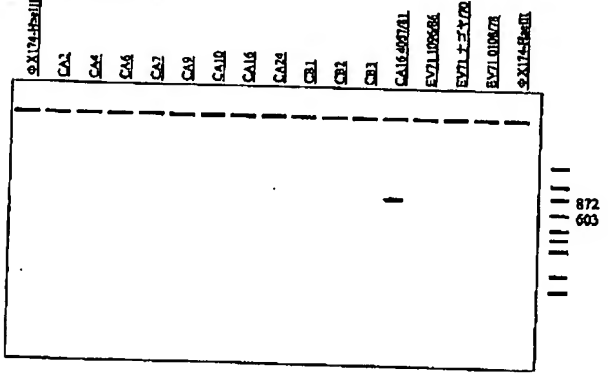
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DRAWINGS

[Drawing 1]



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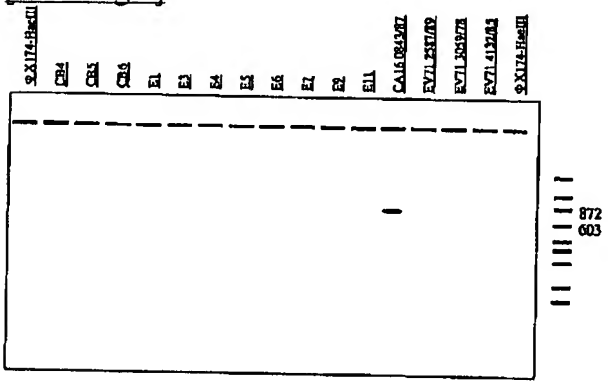

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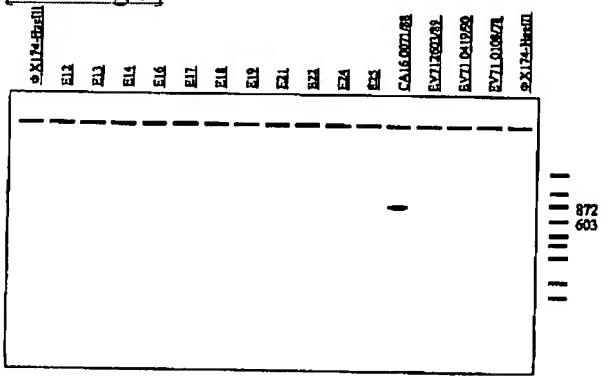

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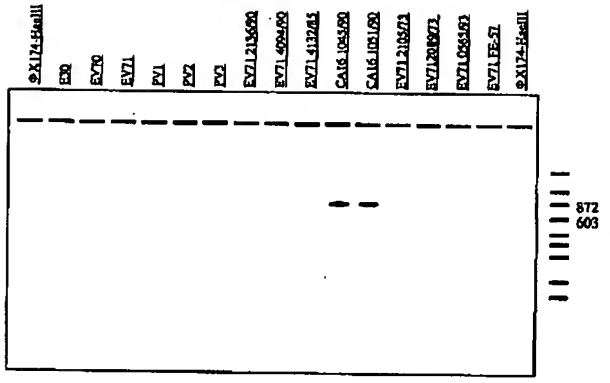

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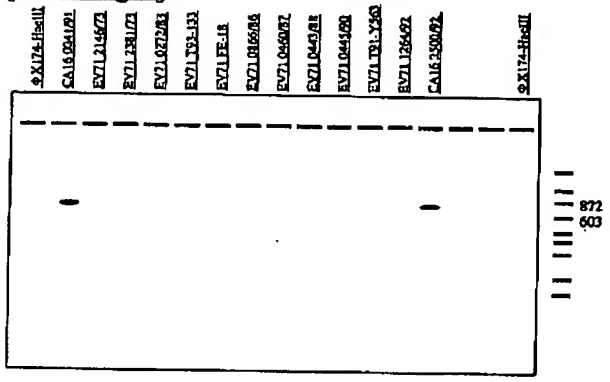

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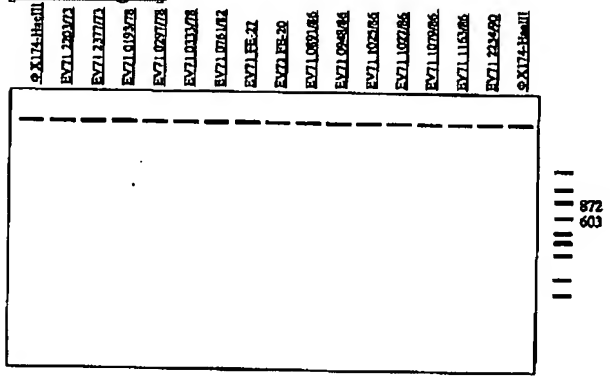

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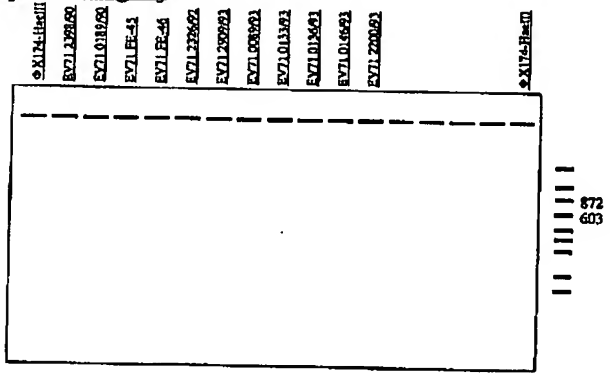

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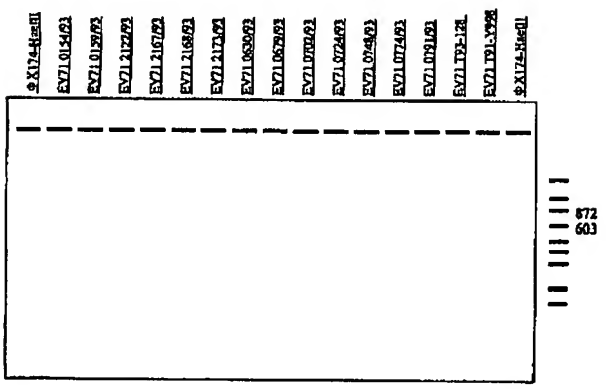

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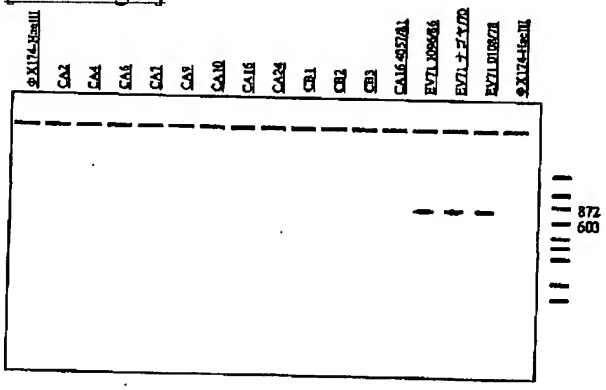
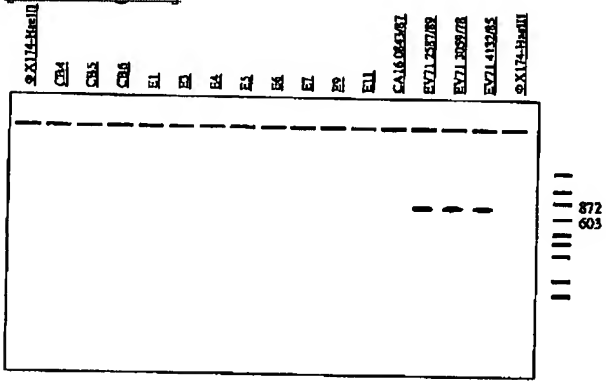
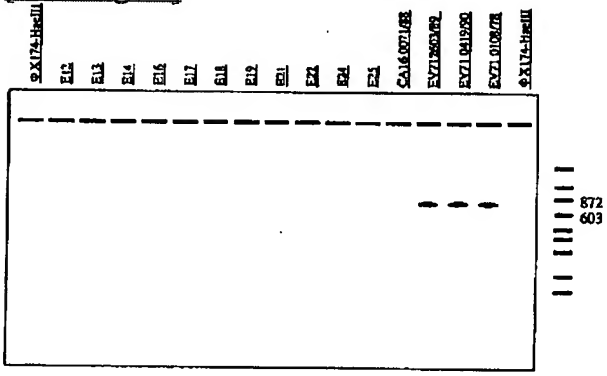


[Drawing 10]



[Drawing 11]



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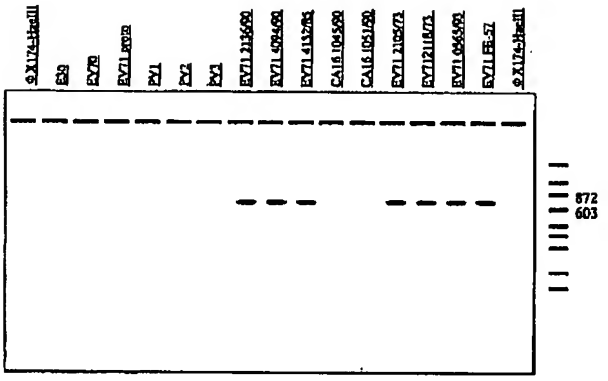
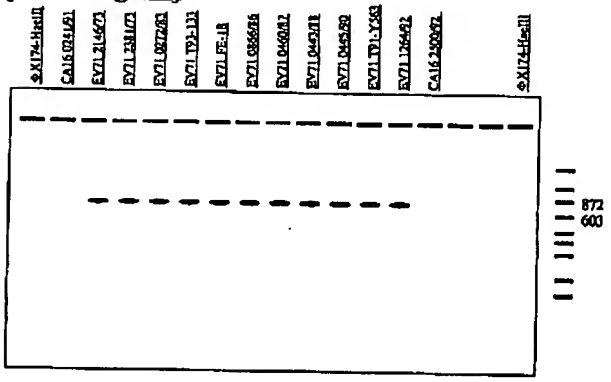
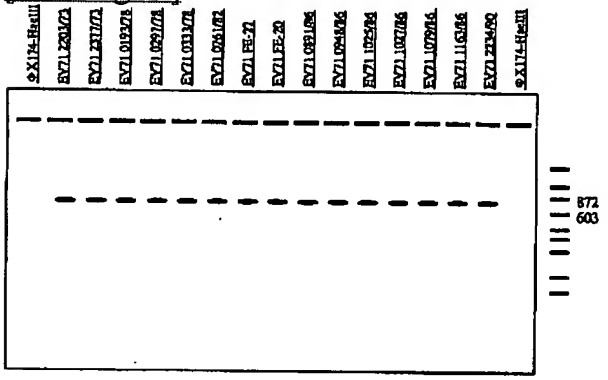


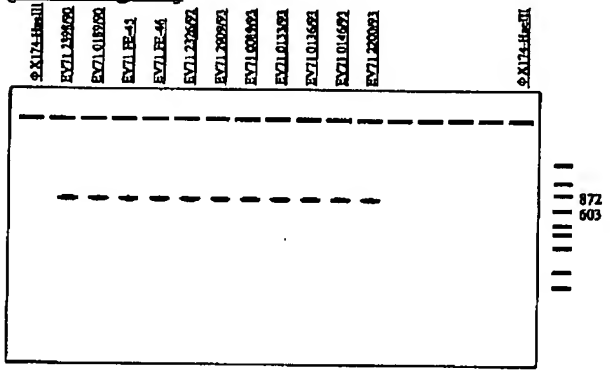
[Drawing 13]



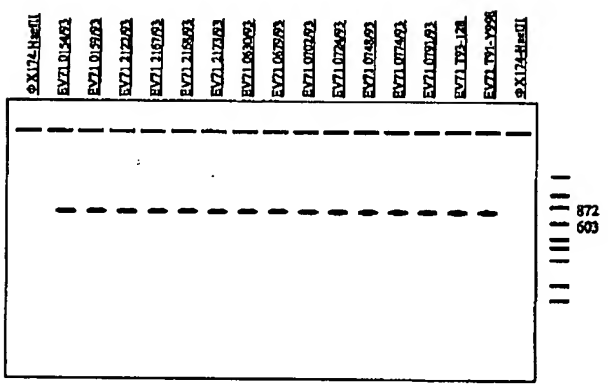
[Drawing 14]



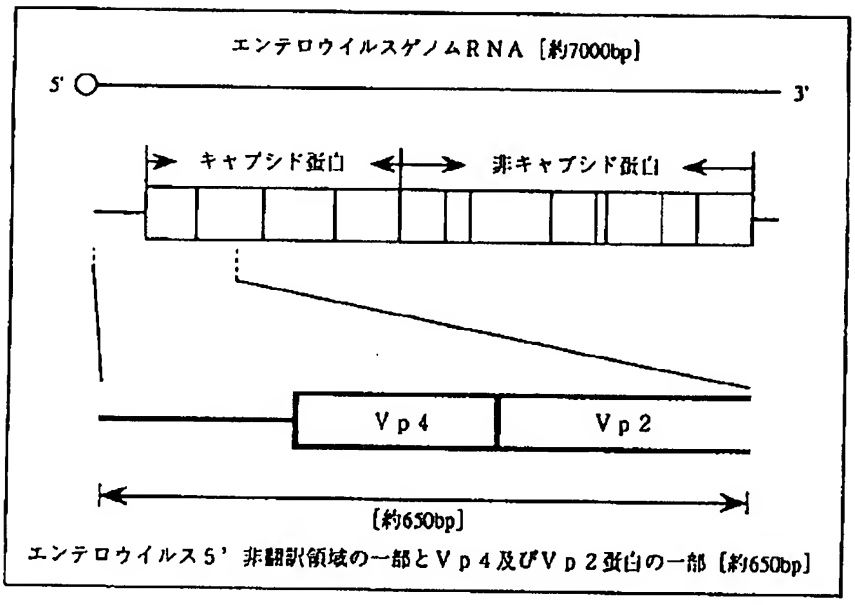
[Drawing 15]



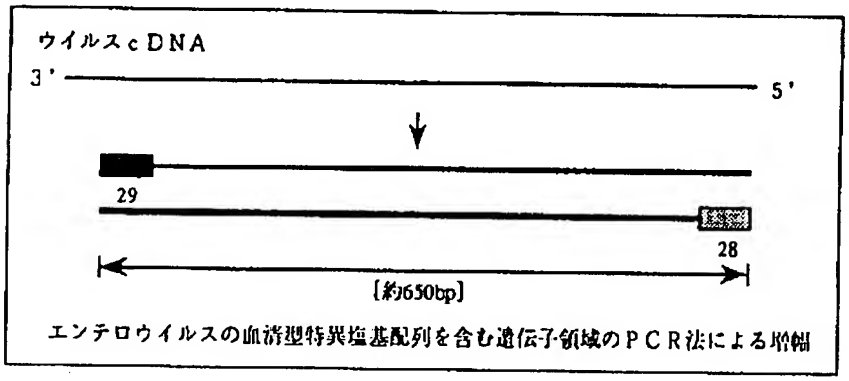
[Drawing 16]



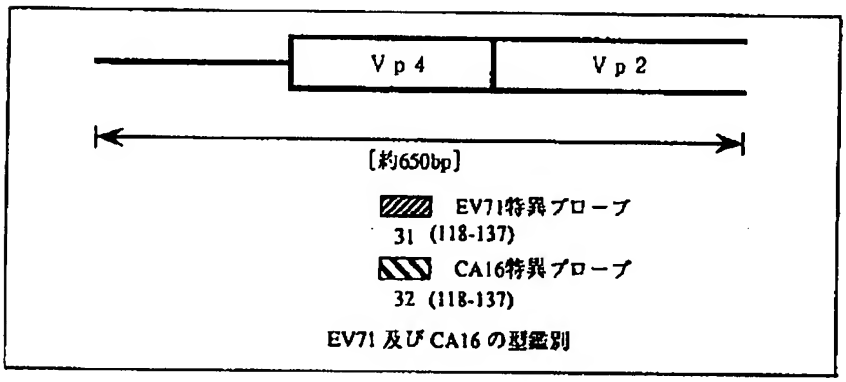
[Drawing 17]



エンテロウイルス５’非翻訳領域の一部とＶｐ４及びＶｐ２蛋白の一部［約650bp］



エンテロウイルスの血清型特異塩基配列を含む遺伝子領域のＰＣＲ法による増幅



EV71及びCA16の型鑑別

[Translation done.]

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最終頁に続く

(54)【発明の名称】　エンテロウイルス71型及びコクサッキーＡ群ウイルス16型の型鑑別方法並びにそれに用いるＤＮＡプローブ及びＤＮＡ断片

(57)【要約】

【目的】　高い精度でエンテロウイルス７１型（ＥＶ７１）とコクサッキーＡ群ウイルス１６型（ＣＡ１６）の型鑑別を可能にする。

【解決手段】　(i)ＥＶ７１及びＣＡ１６の、５′非翻訳領域の一部とエンテロウイルスの各血清型に特異的なＤＮＡ配列を持つＶｐ４及びＶｐ２蛋白の一部をコードするＤＮＡ領域を増幅し、(ii)該増幅ＤＮＡ中のＶｐ４蛋白をコードするＤＮＡ領域のＤＮＡ配列を決定し、(iii)該ＤＮＡ配列中のＥＶ７１及びＣＡ１６の各血清型に特異的なＤＮＡ配列に基き、該ＤＮＡ配列に各々相補性を有するＤＮＡ配列をそれぞれ設計してＤＮＡプローブとし、(iv)該ＤＮＡプローブと上記(i)の方法により得られる増幅ＤＮＡとの結合能を解析することによりＥＶ７１とＣＡ１６の型を鑑別する方法。

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【特許請求の範囲】

【請求項１】　（ｉ）エンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の、５′非翻訳領域の一部とエンテロウイルスの各血清型に特異的なＤＮＡ配列を持つＶｐ４及びＶｐ２蛋白の一部をコードするＤＮＡ領域を増幅し、（ii）該増幅ＤＮＡ中のＶｐ４蛋白をコードするＤＮＡ領域のＤＮＡ配列を決定し、（iii）該ＤＮＡ配列中のエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の各血清型に特異的なＤＮＡ配列に基き、該ＤＮＡ配列に各々相補性を有するＤＮＡ配列をそれぞれ設計してＤＮＡプローブとし、（iv）該ＤＮＡプローブと上記（ｉ）の方法により得られる増幅ＤＮＡとの結合能を解析することを特徴とするエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の型鑑別方法。

【請求項２】　増幅ＤＮＡ中のエンテロウイルス７１型のＶｐ４蛋白をコードするＤＮＡ領域と相補性を有するＤＮＡ配列が、配列番号３１、配列番号３３、配列番号３５、配列番号３７又は配列番号４１で示されるＤＮＡ配列であり、増幅ＤＮＡ中のコクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードするＤＮＡ領域と相補性を有するＤＮＡ配列が、配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９又は配列番号４０で示されるＤＮＡ配列である、請求項１記載の方法。

【請求項３】　配列番号３１、配列番号３３、配列番号３５、配列番号３７又は配列番号４１で示されるＤＮＡ配列を含む、エンテロウイルス７１型のＶｐ４蛋白をコードするＤＮＡ領域の増幅ＤＮＡと相補性を有するＤＮＡプローブ。

【請求項４】　配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９又は配列番号４０で示されるＤＮＡ配列を含む、コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードするＤＮＡ領域の増幅ＤＮＡと相補性を有するＤＮＡプローブ。

【請求項５】　エンテロウイルス７１型のＶｐ４蛋白であって、そのアミノ酸配列中に配列番号１で示されるアミノ酸配列を含む蛋白をコードするＤＮＡ断片。

【請求項６】　コクサッキーＡ群ウイルス１６型のＶｐ４蛋白であって、そのアミノ酸配列中に配列番号２で示されるアミノ酸配列を含む蛋白をコードするＤＮＡ断片。

【発明の詳細な説明】

【０００１】

【発明が属する技術分野】本発明は、エンテロウイルスに属するエンテロウイルス７１型（以下これを「ＥＶ７１」と略記することがある）及びコクサッキーＡ群ウイルス１６型（以下これを「ＣＡ１６」と略記することがある）の鑑別方法、それに用いるＤＮＡプローブ、並びにエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードする遺伝子領域内の塩基配列に関する。

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【０００２】

【従来の技術】ピコルナウイルス科（Picornaviridae）に属するエンテロウイルス（Enterovirus）はおよそ７０種類の血清型に分類されており、多彩な感染症を示すため、臨床症状から原因となるウイルスを推定することは困難である。そのため、病原体を確定するにはウイルスの分離同定が必要となる。しかし、現在のエンテロウイルス分離同定法は、培養法を用いてウイルスを分離し、同定のためには更に中和試験が必要である。そしてこれらのウイルスの分離培養には、２～４週間が必要である。更に、標準株の中和抗血清を使用した中和試験においては、血清型鑑別不可能な分離株が頻繁に出現する。これはエンテロウイルスの遺伝子が自然界ではきわめて高頻で変異をするためと考えられており、これらの解決には常に新鮮分離株を中和する抗血清の作製が必要となる。

【０００３】高感度、特異的にＤＮＡを増幅するポリメラーゼ・チェイン・リアクション法〔Polymerase Chain Reaction 法．以下これを「ＰＣＲ法」と略記する；Saikiら，Science，230巻，p.1350-1354，1985 年参照〕が開発されてから、エンテロウイルスは、５′非翻訳領域の塩基配列に相補的なプライマーを用いたＰＣＲ法や、５′非翻訳領域内、Ｖｐ４とＶｐ２蛋白をコードする遺伝子領域の塩基配列に相補性を有するプライマーを用いたＰＣＲ法で検出されている〔Rotbart，H.，S. J. Clinical Microbiology，28，438-442(1990)；Olive，D. M.，S J. General Virology，71，2141-2147(1990)〕。

【０００４】さらに、ＰＣＲにより増幅されたＤＮＡを用いたストリンジェント・リバース固相ハイブリダイゼーション法によるエンテロウイルスの型同定が報告されている〔石古博昭等，臨床とウイルス，22 巻，ページ 199-207 (1994)〕。しかし、本実験では、同一血清型の中でも年代や地域の違いによりターゲット遺伝子とプローブとの反応性が異なり、特定のウイルス株をプローブに適定することが困難であると予想される。エンテロウイルスによる疾患のなかで、手足口病はその患者から分離されるウイルスがＥＶ７１とＣＡ１６で９０％以上を占めるので、２種の血清型を迅速にかつ簡易に検出することにより手足口病の流行ウイルスを同定することが可能である。

【０００５】

【発明が解決しようとする課題】本発明は、ＥＶ７１とＣＡ１６を高い精度で型鑑別できる方法の提供を目的とする。

【０００６】

【課題を解決するための手段】本発明者らは、上記目的を達成すべく鋭意検討を重ねた結果、ＥＶ７１及びＣＡ

１６のＶｐ４蛋白をコードする遺伝子領域の塩基配列中に、各血清型に特異的な塩基配列があり、該塩基配列を用いてＥＶ７１とＣＡ１６が高い精度で鑑別可能であることを見い出し、本発明を完成するに至った。即ち本発明は、下記１～６に記載のエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の型鑑別方法、それに用いるＤＮＡプローブ及びＤＮＡ断片を提供するものである。

【０００７】１．（ｉ）エンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の、５′非翻訳領域の一部とエンテロウイルスの血清型に特異的なＤＮＡ配列を持つＶｐ４及びＶｐ２蛋白の一部をコードするＤＮＡ領域を増幅し、（ii）該増幅ＤＮＡ中のＶｐ４蛋白をコードするＤＮＡ領域のＤＮＡ配列を決定し、（iii）該ＤＮＡ配列中のエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の各血清型に特異的なＤＮＡ配列に基き、該ＤＮＡ配列に各々相補性を有するＤＮＡ配列をそれぞれ設計してＤＮＡプローブとし、（iv）該ＤＮＡプローブと上記（ｉ）の方法により得られる増幅ＤＮＡとの結合能を解析することを特徴とするエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の型鑑別方法。

【０００８】２．増幅ＤＮＡ中のエンテロウイルス７１型のＶｐ４蛋白をコードするＤＮＡ領域と相補性を有するＤＮＡ配列が、配列番号３１、配列番号３３、配列番号３５、配列番号３７又は配列番号４１で示されるＤＮＡ配列であり、増幅ＤＮＡ中のコクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードする遺伝子領域と相補性を有するＤＮＡ配列が、配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９又は配列番号４０で示されるＤＮＡ配列である、上記１記載の方法。

３．配列番号３１、配列番号３３、配列番号３５、配列番号３７又は配列番号４１で示されるＤＮＡ配列を含む、エンテロウイルス７１型のＶｐ４蛋白をコードするＤＮＡ領域の増幅ＤＮＡと相補性を有するＤＮＡプローブ。

【０００９】４．配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９又は配列番号４０で示されるＤＮＡ配列を含む、コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードするＤＮＡ領域の増幅ＤＮＡと相補性を有するＤＮＡプローブ。

５．エンテロウイルス７１型のＶｐ４蛋白であって、そのアミノ酸配列中に配列番号１で示されるアミノ酸配列を含む蛋白をコードするＤＮＡ断片。

６．コクサッキーＡ群ウイルス１６型のＶｐ４蛋白であって、そのアミノ酸配列中に配列番号２で示されるアミノ酸配列を含む蛋白をコードするＤＮＡ断片。以下、本発明について更に詳細について説明する。

【００１０】本発明の実施においては、特に指示されない限り当該分野の技術範囲内にある分子生物学、微生物学、組換えＤＮＡ、及び免疫学に於ける従来の手法が採用される。このような手法は文献中に詳しく説明されている。例えば次のような文献を参照されたい。Maniatis, Fitsch及び Sambrook, MOLECULAR CLONING ; A LABORATORY MANUAL (1982) ; Kevin struhlら, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, １巻及び２巻；村松正實編、ラボマニュアル遺伝子工学（1990）；村松正實岡山博人編、遺伝子工学ハンドブック、実験医学　別冊（1991）；東京大学医科学研究所制癌研究部編、細胞工学実験プロトコール（1991）。

【００１１】１．エンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の５′非翻訳領域の一部とエンテロウイルスの血清型に特異的な塩基配列を持つＶｐ４及びＶｐ２蛋白の一部をコードする遺伝子領域の増幅

エンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の５′非翻訳領域の一部とエンテロウイルスの血清型に特異的な塩基配列を持つＶｐ４及びＶｐ２蛋白の一部をコードする遺伝子領域（以下これを「エンテロウイルスの血清型特異的塩基配列を含む遺伝子領域」と略称することがある）の増幅は、次のとおり行うことができる。

【００１２】先ず、血清型が既知のエンテロウイルス分離培養株、継代培養されている血清型が既知のエンテロウイルス標準株等から常法によりＲＮＡを抽出し、この抽出ＲＮＡから、逆転写酵素を用いｃＤＮＡを作製する。このｃＤＮＡにエンテロウイルスの血清型特異的塩基配列を含む遺伝子領域の上流の型共通部分及び下流の型共通部分に相補性を有するオリゴヌクレオチドをプライマーとして加えて、エンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型の５′非翻訳領域、Ｖｐ４とＶｐ２をコードする遺伝子領域を含む長さが約６５０塩基の遺伝子ＤＮＡ領域を増幅する。

【００１３】遺伝子の増幅は、通常用いられるＰＣＲ法【このＰＣＲ法の詳細については、特開昭６１－２７４６９７号公報、特開昭６２－２８１号公報、Sakai ら Science 239巻, p.487-491 参照】により容易に行うことができる。エンテロウイルスの血清型特異的塩基配列を含む遺伝子領域の増幅に際して、プライマーとして用いることができるオリゴヌクレオチド（以下これを「血清型共通プライマー」と略称することがある）としては、血清型特異的塩基配列を含む遺伝子領域の上流の型共通部分及び下流の型共通部分に相補性を有するオリゴヌクレオチドを同時に用いるのであれば、いかなるオリゴヌクレオチドであってもよい。それらの中で、好ましくは既知の血清型特異的塩基配列データをもとに、エンテロウイルスに特異的でかつ種間で共通性の高い塩基配列を５′非翻訳領域（上流の型共通部分）とＶｐ２領域（下流の型共通部分）に設定し、その塩基配列に基いて化学合成したオリゴヌクレオチドをプライマーとして用いる

のが適当である。

【００１４】上記プライマーの具体例としては、D.M. Olive et al, Journal of General Virology 71巻、ページ 2141-2147 (1990) に記載されている配列番号２７及び配列番号２８で示されるオリゴヌクレオチド、本願発明者らが先に提案した特開平６－３１１９００号明細書に記載されている配列番号２９及び配列番号３０で示されるオリゴヌクレオチドを挙げることができる。これらプライマーの中で、５´末端側のプライマーとして配列番号２９で示されるオリゴヌクレオチド、３´末端側のプライマーとして配列番号２８で示されるオリゴヌクレオチドを同時に用いるのが適当である。

【００１５】上記したプライマーは、それ自体既知の通常用いられる核酸合成装置、例えばアプライド・バイオシステムズ（Applied Biosystems）社製、モデル３８１－ＡＤＮＡ合成機等を用いる固相合成法により、容易に化学合成することができる。上記増幅ＤＮＡは、常法、例えば、アガロースゲル電気泳動法により分離し、ＤＮＡバンドとして検出することができ、これによりエンテロウイルス７１型及びコクサッキーＡ群ウイルス１６型由来の遺伝子ＤＮＡを確認することができる。

【００１６】２．増幅ＤＮＡ中のＶｐ４蛋白をコードする遺伝子領域の塩基配列の決定

上記増幅ＤＮＡの塩基配列は、例えば、アプライド・バイオシステムズ社製、モデル３７３－Ａ　オートシークエンサーを用い、Dye Deoxy™ ターミネーター法により決定することができる。決定された塩基配列中の翻訳開始コドン及びエンテロウイルスの既知のＶｐ４蛋白及びＶｐ２蛋白を切断するアミノ酸配列に対応する塩基配列【蛋白質　核酸酵素、137巻、14号、ページ 2609-2618 (1992) 参照】を探索することにより、Ｖｐ４蛋白をコードする遺伝子領域の塩基配列を決定することができる。

【００１７】かくして得られるＥＶ７１のＶｐ４蛋白をコードする遺伝子領域の配列を配列番号３～配列番号２１に、そのアミノ酸配列の組合せを配列番号１に示す。また、ＣＡ１６のＶｐ４蛋白をコードする遺伝子領域の配列を配列番号２２～配列番号２６に、そのアミノ酸配列の組合せを配列番号２に示す。上記塩基配列を包含する本発明のＤＮＡ断片は、ＥＶ７１及びＣＡ１６分離株のＲＮＡから合成されたｃＤＮＡのみならず、通常用いられるＤＮＡ合成装置、例えば上記アプライド・バイオシステムズ社製、モデル３８１－Ａ　DNA合成機を用いて合成されたものであってよい。

【００１８】３．ＤＮＡプローブの設計及び合成

エンテロウイルス７１型の特異的な塩基配列に相補性を有するＤＮＡプローブ（以下これを「ＥＶ７１特異プローブ」と略称することがある）の塩基配列は、前記配列番号３～配列番号２１で示される配列中のＥＶ７１に特異的な配列を解析することにより設計することができる。同様に、コクサッキーＡ群ウイルス１６型の特異的な塩基配列に相補性を有するＤＮＡプローブ（以下これを「ＣＡ１６特異プローブ」と略称することがある）の塩基配列は、前記配列番号２２～配列番号２６で示される配列中の、ＣＡ１６に特異的な配列を解析することにより設計することができる。

【００１９】かくして得られるＥＶ７１特異プローブとしては、例えば、配列番号３１、配列番号３３、配列番号３５、配列番号３７又は配列番号４１で示されるオリゴヌクレオチド、ＣＡ１６特異プローブとしては、例えば、配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９又は配列番号４０で示されるオリゴヌクレオチドを挙げることができる。上記した血清型特異プローブのうち、好ましくは、ＥＶ７１特異プローブとして配列番号３１及び配列番号４１、ＣＡ１６特異プローブとして配列番号３２、配列番号３９及び配列番号４０で示されるオリゴヌクレオチドを挙げることができる。上記ＤＮＡプローブは、それ自体既知の通常用いられる核酸合成装置、例えば、アプライド・バイオシステムズ社製、モデル３８１－Ａ　ＤＮＡ合成機等を用いる固相合成法により、容易に化学合成することができる。

【００２０】４．エンテロウイルス７１型及びコクサッキーウイルス１６型の型鑑別

上記した、血清型特異プローブを用いて、次のとおり、ＥＶ７１及びＣＡ１６を鑑別することができる。先ず、診察時に採取した臨床検体からのウイルス分離培養株から、常法によりウイルスＲＮＡを抽出する。該抽出ＲＮＡからｃＤＮＡを合成後、前記血清型共通プライマーを用いて、エンテロウイルスの５´非翻訳領域の一部、Ｖｐ４及びＶｐ２蛋白の一部をコードする遺伝子領域を、前記ＰＣＲ法により増幅する。増幅ＤＮＡの検出は、アガロース電気泳動により増幅ＤＮＡを分離し、適当な染色剤、例えばエチジウム・ブロマイド等で染色後、紫外線照射等により発色させ、そのＤＮＡバンドを確認することにより行うことができる。

【００２１】ＥＶ７１とＣＡ１６の型鑑別は、前記血清型共通プライマーを用いて増幅したＤＮＡ断片と、前記血清型特異ＤＮＡプローブ、すなわちＥＶ７１の検出には、例えば配列番号３１、配列番号３３、配列番号３５、配列番号３７及び配列番号４１で示される配列を、またＣＡ１６の検出には、例えば配列番号３２、配列番号３４、配列番号３６、配列番号３８、配列番号３９及び配列番号４０で示される配列を有するオリゴヌクレオチドを標識することにより作製したＤＮＡプローブとを、固相上で常法によりハイブリダイズさせ、血清型特異ＤＮＡプローブの種類を検出解析することにより行うことができる。

【００２２】固相上へのＤＮＡの結合は、それ自体既知の通常用いられる方法、例えば、増幅ＤＮＡを変性後、

適当な固相、例えばナイロンメンブレンに、常法により化学結合させることにより行うことができるが、好ましくは、増幅ＤＮＡを確認するために使用した上記アガロースゲルを変性後、中和し、例えばナイロンメンブレンに増幅ＤＮＡをトランスファー後固定する方法（以下これを「サザンブロッティング法」と略称することがある）が挙げられる。

【００２３】ＤＮＡプローブの標識は、５′末端のリン酸化反応により〔γ－^{32}Ｐ〕ＡＴＰ、Ｔ４ポリヌクレオチドキナーゼ（東洋紡績社製）を用いて行うことができる。また、標識物の検出は、標識されたＤＮＡプローブとのハイブリダイゼーションを行ったナイロンメンブレンのオートラジオグラフィーを行い、各々のＤＮＡプローブとのハイブリッド形成の有無を調べることにより行うことができる。

【００２４】上記した、本発明で用いられるエンテロウイルスの血清型特異的ＤＮＡ配列を含む遺伝子領域、即ち、エンテロウイルスの５′非翻訳領域の一部とＶｐ４及びＶｐ２蛋白の一部をコードする領域、血清型共通プライマー、ＤＮＡプローブ、該プライマーを用いて増幅＊可能なＤＮＡ断片等の一例を、図１７に示す。図中、２桁の数字は配列番号、（　）内の数字はＤＮＡプローブが結合するＶｐ４蛋白をコードする遺伝子領域の位置（塩基対番号）、〔　〕内の数字は増幅されるＤＮＡ断片の大きさ、ｂｐは塩基対を示す。

【００２５】

【実施例】次に実施例を挙げて本発明をさらに詳細に説明するが、下記の実施例は本発明について具体的な認識を得る一助としてのみ挙げたものであり、これによって本発明の範囲は何等限定されるものではない。

【００２６】実施例１　ＥＶ７１とＣＡ１６分離培養株のシークエンス

（Ａ）使用微生物

下記の、患者より分離され特異抗血清を用いた中和試験により血清型が同定されたエンテロウイルス分離株及び標準株を用いて実験を行った。ここで用いた標準株は、国立予防衛生研究所において維代培養されているウイルス標準株である。

【００２７】

（１）エンテロウイルス分離株

株名（血清型）	分離時期
コクサッキーＡ群ウイルス１６型（ＣＡ１６）	
１５４７／７９	１９７９年
４０５７／８１	１９８１年
０２１６／８６	１９８６年
０２４１／９１	１９９１年
エンテロウイルス７１型（ＥＶ７１）	
ナゴヤ／７０	１９７０年
０１０８／７８	１９７８年
３０５９／７８	１９７８年
３３５９／８３	１９８３年
４１３２／８５	１９８５年
Ｔ８６２３６ａ／８６	１９８６年
１０９１／８９	１９８９年
０４４５／９０	１９９０年
０００４／７８	１９７８年
１０９６／８６	１９８６年
０８７２／８９	１９８９年
２５８７／８９	１９８９年
２６０３／８９	１９８９年
０３７５／９０	１９９０年
２１３６／９０	１９９０年
２３９８／９０	１９９０年
４０９４／９０	１９９０年
０４１９／９０	１９９０年

【００２８】（２）標準株

株名（血清型）	分離時期
コクサッキーＡ群ウイルス	１６型（ＣＡ１６）
エンテロウイルス	７１型（ＥＶ７１）

【００２９】（Ｂ）ＲＮＡの抽出

上記ウイルス液から、スマイテストＲキット（住友金属社製）にてＲＮＡを抽出し、イソプロパノール沈殿を行った。

（C）cDNAの合成
上記（B）項で得たRNAを鋳型としてリバーストランスクリプターゼ（GIBCO/BRL社製）を用いて、各ウイルスに由来するcDNAを合成した。
（D）PCR用のプライマーの合成
配列番号28及び配列番号29で示されるオリゴヌクレオチドをホスホアミダイド（Phosphoamidide）法により、アプライド・バイオシステムズ社製、モデル381－A DNA合成機を用いて合成した。上記オリゴヌクレオチドをOPC™カートリッジを用いて精製し、PCRのプライマーとして使用した。
【0030】（E）標的DNAの増幅（PCR法）
反応液は、終濃度を10mM Tris・HCl（pH8.0）、50mM KCl、0.001%ゼラチン、0.5mM $MgCl_2$、0.05mM dNTPs、2.5%ホルムアミドとした溶液に、上記オリゴヌクレオチド合成プライマー（配列番号28及び配列番号29；各50μM）各0.1μl、前記（C）項で合成されたエンテロウイルスcDNA 100ng～1μg、及びTaqポリメラーゼ（日本ロッシュ社製）0.125μl（0.625U）を加え、計50μlとしたものを調製した。PCRは、95℃30秒、55℃30秒の反応条件で、14サイクルでアンプリフィケーションシステム（Amplification System；シータス社）を用いて標的DNAを増幅した。
【0031】さらに、上記溶液に、終濃度を10mM Tris・HCl（pH8.0）、50mM KCl、0.001%ゼラチン、2.35mM $MgCl_2$、0.15mM dNTPs、2.5%ホルムアミドとした溶液に、上記オリゴヌクレオチド合成プライマー（配列番号28及び配列番号29；各50μM）各1.0μl、Taqポリメラーゼ0.25μl（1.25U）を加えた計50μlの溶液を加え、95℃30秒、55℃30秒、72℃45秒、40サイクルで増幅した。

【0032】（F）ゲル電気泳動法による増幅DNAの確認
3.0%のアガロースゲルにエチジウムブロマイドを0.5μg/ml加え、上記（E）項で増幅したDNAの電気泳動を行った。泳動後、254nmの紫外線を照射し、エチジウムブロマイドの発色反応によりDNAバンドを検出し、エンテロウイルスの5′非翻訳領域の一部、Vp4とVp2蛋白の一部をコードする遺伝子領域に由来する約650塩基の標的DNAバンドを検出した。
【0033】（G）Vp4領域の塩基配列の決定
上記（F）で確認されたPCR産物は、Centricon-100（アミコン社製）を用いて精製後、Dye Deoxy™ ターミネーター法によりCycle Sequencing反応を行い、アプライド・バイオシステムズ社製モデル373－A DNAシークエンサーを用い、塩基配列を決定した。決定された塩基配列中の翻訳開始コドン及びエンテロウイルスの既知のVp4蛋白及びVp2蛋白を切断するアミノ酸配列に対応する塩基配列を探索することにより、Vp4蛋白をコードする遺伝子領域の塩基配列を決定した。かくして得られたEV71のVp4蛋白をコードする配列を配列番号3～配列番号21に、CA16のVp4蛋白をコードする配列を配列番号22～配列番号26に示す。
【0034】実施例2 EV71及びCA16の型鑑別
（A）使用微生物
（1）標準株
国立予防衛生研究所において維代培養されている下記39種類の血清型エンテロウイルス標準株を用いて実験を行った。これらのエンテロウイルスは、いずれも特異抗血清を用いた中和試験で血清型が同定されている標準株である。
【0035】

株名（血清型）		略号
コクサッキーA群ウイルス	2型	CA2
〃	4〃	CA4
〃	6〃	CA6
〃	7〃	CA7
〃	9〃	CA9
〃	10〃	CA10
〃	16〃	CA16
〃	24〃	CA24
コクサッキーB群ウイルス	1型	CB1
〃	2〃	CB2
〃	3〃	CB3
〃	4〃	CB4
〃	5〃	CB5
〃	6〃	CB6
エコーウイルス	1型	E1
〃	3〃	E3

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〃	7〃	E7
〃	9〃	E9
〃	11〃	E11
〃	12〃	E12
〃	13〃	E13
〃	14〃	E14
〃	16〃	E16
〃	17〃	E17
〃	18〃	E18
〃	19〃	E19
〃	21〃	E21
〃	22〃	E22
〃	24〃	E24
〃	25〃	E25
〃	30〃	E30
エンテロウイルス	70型	EV70
〃	71〃	EV71
ポリオウイルス	1型	PV1
〃	2〃	PV2
〃	3〃	PV3

【0036】（2）エンテロウイルス分離株
下記の、患者より分離され特異抗血清を用いた中和試験により血清型が同定されたエンテロウイルス分離株を用＊いて実験を行った。

【0037】

株名	分離時期
コクサッキーA群ウイルス16型（CA16）	
4057／81	1981年
0843／87	1987年
0071／88	1988年
1045／90	1990年
1051／90	1990年
0241／90	1990年
2500／92	1992年
エンテロウイルス71型（EV71）	
ナゴヤ／70	1970年
2105／73	1973年
2089／73	1973年
2146／73	1973年
2203／73	1973年
2377／73	1973年
2381／73	1973年
0108／78	1978年
3059／78	1978年
0193／78	1978年
0297／78	1978年
0333／78	1978年
0761／82	1982年
FE－27	1982年

13	14
0272／83	1983年
FE－18	1983年
FE－20	1983年
4132／85	1985年
0866／86	1986年
0891／86	1986年
0948／86	1986年
1025／86	1986年
1027／86	1986年
1079／86	1986年
1096／86	1986年
1163／86	1986年
0460／87	1987年
0443／88	1988年
2587／89	1989年
2603／89	1989年
2136／90	1990年
2234／90	1990年
2398／90	1990年
0419／90	1990年
0445／90	1990年
0189／90	1990年
4094／90	1990年
FE－45	1990年
FE－46	1990年
T91－Y563	1991年
1264／92	1992年
2326／92	1992年
2909／93	1993年
0089／93	1993年
0133／93	1993年
0136／93	1993年
0146／93	1993年
2200／93	1993年
0154／93	1993年
0159／93	1993年
2122／93	1993年
2167／93	1993年
2168／93	1993年
2173／93	1993年
0565／93	1993年
0630／93	1993年
0679／93	1993年
0702／93	1993年
0724／93	1993年
0748／93	1993年
0774／93	1993年
0791／93	1993年
FE－57	1993年
T93－128	1993年

15 16

T93－133
T93－Y998

【００３８】（Ｂ）ＲＮＡ抽出とｃＤＮＡ合成．及びエンテロウイルス遺伝子のＰＣＲによる増幅とその検出
実施例１の（Ｂ）～（Ｆ）項で示したように、上記エンテロウイルス標準株及び分離株のＲＮＡを抽出し．５′非翻訳領域の一部、Ｖｐ４とＶｐ２蛋白の一部に対応する部分を増幅後．アガロースゲルを用いた電気泳動にて増幅を確認した。なお、マーカーとしてφＸ１７４ファージのＨａｅIII 分解物を同時に泳動した。

【００３９】（Ｃ）サザンブロット法
実施例２の（Ｂ）項で増幅ＤＮＡの確認のために使用したアガロースゲルを、アルカリ変性液（０．５Ｍ ＮａＯＨ，１．５Ｍ ＮａＣｌ）で３０分間処理後、中和液（１Ｍ Ｔｒｉｓ・ＨＣｌ，１．５Ｍ ＮａＣｌ）で３０分間入れゲルを中性に戻し、ＤＮＡを変性させた。さらに、常法により、サザンブロッティングを一晩行い、ナイロンメンブレンに移行したＤＮＡを２５４nmの紫外線１．２×１０５μJOULES/cm² （UV stratalinker™ ；ストラタジーン製）により固着させた。

【００４０】（Ｄ）血清型特異的プローブの設計と合成
実施例１（Ｇ）項で示したＣＡ１６及びＥＶ７１のＶｐ４蛋白質をコードするＤＮＡ領域の塩基配列［配列番号３から配列番号２６］を解析し、これらの配列からＥＶ７１特異プローブを配列番号３１及び配列番号４１、ＣＡ１６特異プローブを配列番号３２．配列番号３９及び配列番号４０のように設計し、実施例１（Ｄ）項に準じて合成した。上記ＤＮＡプローブの標識は、５′末端のリン酸化反応により［γ－³²Ｐ］ＡＴＰ、Ｔ４ポリヌクレオチドキナーゼ（東洋紡績社製）を用いて行った。

【００４１】（Ｅ）ハイブリダイゼーション
上記実施例２（Ｃ）項で作製したメンブレンは．反応液として１．０Ｍ ＮａＣｌ．５０mＭ Ｔｒｉｓ・ＨＣｌ（pH７．５）．０．５％ＰＶＰ、０．２％ヘパリン、１mＭ ＥＤＴＡ．２％ＳＤＳを用い、５０℃で１時間処理し．プレハイブリダイゼーションを行った。ハイブリダイゼーションは．上記ハイブリダイゼーション溶液に、実施例２の（Ｄ）項で合成した［γ－³²Ｐ］ＡＴＰ標識ＤＮＡプローブを、１０⁶ cpm/ml加え．５０℃．４時間反応させた。メンブレンを５×ＳＳＣ－０．１％ＳＤＳ溶液中で、５０℃で５分．２回洗浄後．２×ＳＳＣ－０．１％ＳＤＳ溶液中で．５０℃で５分、２回洗浄し、非特異的に結合しているプローブを取り除いた。ナイロンメンブレンを乾燥後、ラップに包み．オートラジオグラフィーを－８０℃で４５分間、さらに室温で１５分間行った後、現像した。

【００４２】かくして得られた、ＣＡ１６特異的ＤＮＡプローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッドの形成を図１～図８に．ＥＶ７１特異的ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を図９～図１６に示す。図から明らかなとおり、ＥＶ７１特異的ＤＮＡプローブにはすべてのＥＶ７１分離株が、ＣＡ１６特異的ＤＮＡプローブにはすべてのＣＡ１６分離株が反応し、かつ、血清型特異的ＤＮＡプローブと各血清型のエンテロウイルス標準株から得られたＤＮＡとの間での交差反応はなく、これは特異的な結合であることが判明した。上記のとおり．本発明の方法によれば、ＣＡ１６とＥＶ７１が高精度に鑑別可能である。

【００４３】
【配列表】
配列番号：１
配列の長さ：69
配列の型：アミノ酸
トポロジー：直鎖状
他の情報
下記配列中、アミノ酸番号３番目のGqq は、Ser 又はThr を示し、アミノ酸番号７番目のHhh は、Ala 又はThr を示し、アミノ酸番号９番目の Iiiは．Arg 又は Xaaを示し．アミノ酸番号１７番目のJjj は．Asn 又は Xaaを示し．アミノ酸番号３１番目のKkk は．Asn 又はXaa を示し．アミノ酸番号３４番目のLll は．Lys 又はXaa を示し．アミノ酸番号３５番目のMmm は．Asp 又はXaa を示し．アミノ酸番号４３番目のNnn は．Lys 又はXaa を示し．アミノ酸番号４７番目のOoo は．Lys 又はXaa を示し．アミノ酸番号４９番目のPpp は．Asp 又はXaa を示し．アミノ酸番号５３番目のQqq は．Phe 又はXaa を示し．アミノ酸番号５４番目のRrr は．Ala 又はGly 又はXaa を示し．アミノ酸番号５５番目のSss は．Asn 又はXaa を示す。

配列
Met Gly Gqq Gln Val Ser Hhh Gln Iii Ser Gly Ser His Glu Asn Ser
5 10 15
Jjj Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr
20 25 30
Tyr Lll Mmm Ser Tyr Ala Ala Thr Ala Gly Nnn Gln Ser Leu Ooo Gln
35 40 45
Ppp Pro Asp Lys Qqq Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60

17 18

Ala Ala Pro Leu Lys
65

【００４４】配列番号：２
配列の長さ：69
配列の型：アミノ酸
トポロジー：直鎖状
他の情報
下記配列中、アミノ酸番号９番目のAaa は、Arg 又はXa* を示す。

配列

Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser
5 10 15
Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr
20 25 30
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
35 40 45
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60
Ala Pro Pro Leu Lys
65

＊a を示し、アミノ酸番号１９番目のBbb は、Ala 又はXa
a を示し、アミノ酸番号２１番目のCcc は、Glu 又はXa
a を示し、アミノ酸番号２３番目のDdd は、Ser 又はTh
r を示し、アミノ酸番号２４番目のEee は、Thr 又はXa
a を示し、アミノ酸番号２９番目のFffは、Thr 又はPro

【００４５】配列番号：３
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus enterovirus type 71

※

20※株名：BrCr
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

```
ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT GAG AAT TCC     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAC TCA GCC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAC AAA GAC TCG TAT GCT GGC ACT GCT GGA AAG CAA AGT CTC AAA CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCA GCG CCC TTA AAG                                             207
Ala Ala Pro Leu Lys
65
```

【００４６】配列番号：４
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源

生物名：enterovirus type 71
株名：ナゴヤ／７０
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報

19 20

エンテロウイスル７１型のＶｐ４蛋白をコードする遺伝＊ ＊子領域の１～２０７番目の塩基配列

配列

```
ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCC     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAC AAG GAC TCT TAT GCT GGC ACA GCA GGC AAG CAG AGC CTC AAA CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAC CCT GAC AAG TTT GGC AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG    192
Asp Pro Asp Lys Phe Gly Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCG GGG CCA CTG AAG                                             207
Ala Ala Pro Leu Lys
 65
```

【００４７】配列番号：５

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

※株名：０１０８／７８

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝

※ 子領域の１～２０７番目の塩基配列

配列

```
ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAT TCA GCT ACA GAA GGC TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAC AAG GAC TCT TAT GCT GGC ACA GCA GGT AAG CAG AGC CTC AAA CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCC GGG CCA CTG AAG                                             207
Ala Ala Pro Leu Lys
 65
```

【００４８】配列番号：６

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：ｅｎｔｅｒｏｖｉｒｕｓ ｔｙｐｅ ７１ ★

★株名：３０５９／７３

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝

子領域の１～２０７番目の塩基配列

配列

```
     21                                                        22
ATG GGT TCA CAA GTG TCT ACT CAG CGG
TCC GGC TCC CAC GAG AAT TCT     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAC AAG GAC TCT TAT GCT GGC ACA GCA GGC AAG CAG AGC CTT AAA CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCC GGG CCA CTG AAG                                            207
Ala Ala Pro Leu Lys
 65
```

【００４９】配列番号：７

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71 ＊

＊株名：０００４／７８
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

```
ATG GGA TCG CAG GTG TCC ACA CAA CGC TCT GGT TCG CAT GAA AAT TCT     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAT TCA GCC ACT GAA GGT TCC ACT ATA AAC TAC ACC ACC ATC AAT TAC     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAT AAG GAC TCT TAT GGC GCT ACA GCA GGC AAA CAG AGC CTT AAG CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAT CCA GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCG GCA CCA CTG AAA                                            20
7
Ala Ala Pro Leu Lys
 65
```

【００５０】配列番号：８

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：ｃＤＮＡ　ｔｏ　ｇｅｎｏｍｉｃ　ＲＮＡ
起源
生物名：enterovirus type 71 ※

※株名：３３５９／８３
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

```
      23                                                        24
ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT    48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACT ATC AAC TAT    96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAC AAG GAC TCT TAT GCT GGC ACA GCA GGC AAA CAG AGC CTC AAA CAA   144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
        35                  40                  45
GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG   192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCG GGC CCG CTG AAG                                               207
Ala Ala Pro Leu Lys
 65
```

【００５１】配列番号：９

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

＊株名：４１３２／８５

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

＊

配列

```
ATG GGC TCA CAA GTG TCT ACT CAG CGA TCC GGC TCC CAC GAG AAT TCC    48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC ANC TAT    96
Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr
            20                  25                  30
TAC ANA GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT AMA CAA   144
Tyr Xaa Xaa Ser Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln
        35                  40                  45
GNC CCT GAT AAG TKT GCT AMT CCT GTC AAG GAC ATT TTC ACT GAA ATG   192
Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp Ile Phe Thr Glu Met
    50                  55                  60
GCC GGG CCA CTA AAA                                               207
Ala Ala Pro Leu Lys
 65
```

【００５２】配列番号：１０

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

※株名：Ｔ８６２３６ａ

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

※

配列

```
ATG GGT ACA CAA GTA TCC ACT CAG SGG TCC GGC TCC CAC GAG AAT TCT    48
Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser
```

```
                 5                   10                  15
AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
             20                  25                  30
TAC AAG GAC TCT TAT GCT GCT ACA GCA GGC AAA CAG AGC CTC AAA CAA    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
         35                  40                  45
GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTT ACT GAA ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
     50                  55                  60
GCG GCG CCA CTG AAG                                                207
Ala Ala Pro Leu Lys
             65
```

【0053】配列番号：11
配列の長さ：207
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71
株名：1096／86
配列の特徴
特徴を表す記号：CDS
存在位置：1－207
特徴を決定した方法：E
他の情報
エンテロウイルス71型のVp4蛋白をコードする遺伝子領域の1～207番目の塩基配列
配列

```
ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                 5                   10                  15
AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC     96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
             20                  25                  30
TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG    144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
         35                  40                  45
GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG    192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
     50                  55                  60
GCA GCG CCA CTA AAG                                                207
Ala Ala Pro Leu Lys
 65
```

【0054】配列番号：12
配列の長さ：207
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71
株名：1091／89
配列の特徴
特徴を表す記号：CDS
存在位置：1－207
特徴を決定した方法：E
他の情報
エンテロウイルス71型のVp4蛋白をコードする遺伝子領域の1～207番目の塩基配列
配列

```
ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC CAC GAG AAT TCC     48
Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser
                 5                   10                  15
AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT     96
```

27 28

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30

TAC AAA GAC TCT TAT GCT GCA ACA GCA GGC AAA CAG AGC CTC AAA CAA 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45

GAC CCT GAT AAG TTT GCT AAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60

GCT GGG CCA CTG AAG 207
Ala Ala Pro Leu Lys
65

【００５５】配列番号：１３
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：ｅｎｔｅｒｏｖｉｒｕｓ　ｔｙｐｅ　７１　＊

＊株名：０８７２／８９
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC
TCC GGT TCG CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15

AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30

TAC AAG GAC TCC TAT GCT GGC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45

GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60

GCA GGG CCA CTA AAG 207
Ala Ala Pro Leu Lys
65

【００５６】配列番号：１４
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71

※株名：２５８７／８９
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15

AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

29 30

20 25 30

TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45

GAT CCA GAT AAG TTT GCA AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60

GCA GGG CCA CTA AAG 207
Ala Ala Pro Leu Lys
65

【００５７】配列番号：１５

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：ｃＤＮＡ ｔｏ ｇｅｎｏｍｉｃ ＲＮＡ
起源
生物名：enterovirus type 71 ＊

＊株名：２６０３／８９
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白を
子領域の１～２０７番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15

AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30

TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45

GAT CCA GAT AAG TTT GSA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Xaa Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60

GCA GGG CCA CTA AAG 207
Ala Ala Pro Leu Lys
65

【００５８】配列番号：１６

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状

※株名：０４４５／９０
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ

31 32

TAC AAA GAC TCT TAT GCT GCA ACA GCA GGC AAA CAG AGC CTC AAA CAA 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45
GAC CCT GAT AAG TTT GCT AAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60
GCT GGG CCA CTG AAG 207
Ala Ala Pro Leu Lys
65

【００５９】配列番号：１７

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15
AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30
TAC AAG GAC TCC TAT GCT GGC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45
GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60
GCA GGG CCA CTA AAG 207
Ala Ala Pro Leu Lys
65

10＊株名：０３７５／９０
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白を
＊　子領域の１～２０７番目の塩基配列

【００６０】配列番号：１８

配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源

※株名：２１３６／９０
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
40 エンテロウイルス７１型のＶｐ４蛋白を

35 40 45

GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192

Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GCA GCG CCA CTA AAG 207

Ala Ala Pro Leu Lys

65

【0061】配列番号：１９

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

＊株名：２３９８／９０

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列 ＊

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA CAT GAA AAC TCT 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

10

AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 30

TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAG AGC CTC AAG CAG 144

Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln

40

GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192

Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 60

GCA GCG CCA CTA AAG 207

Ala Ala Pro Leu Lys

65

【0062】配列番号：２０

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：ｅｎｔｅｒｏｖｉｒｕｓ ｔｙｐｅ ７１

※株名：４０９４／９０

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

エンテロウイルス７１型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列 ※

配列

35 36

GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60
GCA GGG CCA CTA AAA 207
Ala Ala Pro Leu Lys
65

【００６３】配列番号：２１
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：enterovirus type 71

＊株名：０４１９／９０
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
エンテロウイルス７１型のＶｐ４蛋白を
＊ 子領域の１～２０７番目の塩基配列

配列
ATG GGC TCA CAG GTG TCC ACG CAA CGC TCC GGC TGG CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15
AAT TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30
TAC AAG GAC TCC TAT GCC GGC ACA GCA GGC AAA CAG AGT CTT AAG CAG 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
35 40 45
GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
50 55 60
GCT GGG CCA CTA AAG 207
Ala Ala Pro Leu Lys
65

【００６４】配列番号：２２
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：ｃＤＮＡ ｔｏ ｇｅｎｏｍｉｃ ＲＮＡ
起源
生物名：entrovirus coxsackievirus A16

※株名：G10
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
コクサッキーＡ群ウイルス１６型のＶｐ
※ する遺伝子領域の１～２０７番目の塩基
配列

37 38
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60
GCT CCT CCC TTG AAA 207
Ala Pro Pro Leu Lys
65

【００６５】配列番号：２３ ＊株名：１５４７／７９
配列の長さ：２０７ 配列の特徴
配列の型：核酸 特徴を表す記号：ＣＤＳ
鎖の数：一本鎖 存在位置：１－２０７
トポロジー：直鎖状 10 特徴を決定した方法：Ｅ
配列の種類：cDNA to genomic RNA 他の情報
起源 コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコード
生物名：coxsackievirus A16 ＊ する遺伝子領域の１～２０７番目の塩基配列

配列
ATG GGG TCA CAG GTT TCC ACT CAG CGG TCT GGG TCA CAT GAG AAC TCA 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15
AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA ACC ATA AAT TAC 96
Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30
TAT AAG GAT GCA TAT GCT GCA AGT GGG GGG CGC CAG GAT ATG TCC CAA 144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
35 40 45
GAC CGG AAG AAA TTT ACC GAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60
GCT CCA CCA CTT AAA 207
Ala Pro Pro Leu Lys
65

【００６６】配列番号：２４ 30※株名：４０５７／８１
配列の長さ：２０７ 配列の特徴
配列の型：核酸 特徴を表す記号：ＣＤＳ
鎖の数：一本鎖 存在位置：１－２０７
トポロジー：直鎖状 特徴を決定した方法：Ｅ
配列の種類：cDNA to genomic RNA 他の情報
起源 コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコード
生物名：coxsackievirus A16 ※ する遺伝子領域の１～２０７番目の塩基配列

配列
ATG GGG TCA CAG GTC TCC ACT CAG CGG TCT GGG TCA CAT GAG AAC TCA 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15
AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96
Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30
TAT AAG GAT GCA TAT GCT GCA AGT GGG GGG CGC CAG GAT ATG TCC CAA 144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
35 40 45
GAC CGG AAG AAA TTT ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60

39 40

GCT CCA CCA CTC AAA 207

Ala Pro Pro Leu Lys

65

【００６７】配列番号：２５

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：coxsackievirus A16

＊株名：０２１６／８６

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

＊ コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

```
ATG GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG TCA CAC GAA AAC TCA     48
Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAC TCT GYA TCG GRG GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC     96
Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr Pro Ile Asn Tyr
            20                  25                  30
TAT AAG GAT GCA TAT GCT GCA AGT GCG GGA CGM CAG GAT ATG TCC CAG    144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
        35                  40                  45
GAC CCG AAG AAA TTC ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG    192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
    50                  55                  60
GCT CCA CCG CTC AAA                                             207
Ala Pro Pro Leu Lys
65
```

【００６８】配列番号：２６

配列の長さ：２０７

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：coxsackievirus A16

※株名：０２４１／９１

配列の特徴

特徴を表す記号：ＣＤＳ

存在位置：１－２０７

特徴を決定した方法：Ｅ

他の情報

※ コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコードする遺伝子領域の１～２０７番目の塩基配列

配列

```
ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT GGG TCA CAT GAG AAC TCA     48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
                5                  10                  15
AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC     96
Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
            20                  25                  30
TAT AAA GAT GCA TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA    144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
        35                  40                  45
GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG    192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
    50                  55                  60
GCT CCA CCA CTC AAA                                             207
Ala Pro Pro Leu Lys
```

39 40
GCT CCA CCA CTC AAA 207
Ala Pro Pro Leu Lys
65

【００６７】配列番号：２５
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：coxsackievirus A16

＊株名：０２１６／８６
配列の特徴
特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
10 コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコード
＊ する遺伝子領域の１～２０７番目の塩基配列

配列
ATG GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG TCA CAC GAA AAC TCA 48
Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser
5 10 15
AAC TCT GYA TCG GRG GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC 96
Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr Pro Ile Asn Tyr
20 25 30
TAT AAG GAT GCA TAT GCT GCA AGT GCG GGA GCM CAG GAT ATG TCC CAG 144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
35 40 45
GAC CCG AAG AAA TTC ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60
GCT CCA CCG CTC AAA 207
Ala Pro Pro Leu Lys
65

【００６８】配列番号：２６
配列の長さ：２０７
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：cDNA to genomic RNA
起源
生物名：coxsackievirus A16

※株名：０２４１／９１
配列の特徴
30 特徴を表す記号：ＣＤＳ
存在位置：１－２０７
特徴を決定した方法：Ｅ
他の情報
コクサッキーＡ群ウイルス１６型のＶｐ４蛋白をコード
※ する遺伝子領域の１～２０７番目の塩基配列

配列
ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT GGG TCA CAT GAG AAC TCA 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
5 10 15
AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96
Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
20 25 30
TAT AAA GAT GCA TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA 144
Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln
35 40 45
GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG 192
Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met
50 55 60
GCT CCA CCA CTC AAA 207
Ala Pro Pro Leu Lys

65

【００６９】配列番号：２７
配列の長さ：１６
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
CTACTTTGGG TGTCGG

【００７０】配列番号：２８
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
GGTAAQTTCC ACCACCANCC

【００７１】配列番号：２９
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
CATCTTTGGG TGTCGGTGTT

【００７２】配列番号：３０
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
TCAGGCAACT TCCACCACCA

【００７３】配列番号：３１
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
AGGCTCTGTT TGCCTGCTGT

【００７４】配列番号：３２
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
ATATCCTGGC GCCCCGCACT

【００７５】配列番号：３３
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列

＊配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスの５´非翻訳領域の一部で antigenom
ic sense RNAに相補性を有する配列
＊

16

※他の情報
エンテロウイルスのＶｐ２蛋白をコードする遺伝子領域
の一部で genomic sense RNAに相補性を有する配列。配
列中．塩基番号６番目のＱはＴ又はＣを示し、塩基番号
１８番目のＮはＡ又はＣ又はＧ又はＴを示す。
※

20

★配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスの５´非翻訳領域の一部で antigenom
ic sense RNAに相補性を有する配列
★

２０

☆配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶＰ２蛋白をコードする遺伝子領域
の一部で　ｇｅｎｏｍｉｃ　ｓｅｎｓｅ　ＲＮＡに相補
☆　性を有する配列

20

◆配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域
の１１８～１３７番目の antigenomic sense RNAに相補
◆　性を有する配列

20

＊配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域
の１１８～１３７番目の antigenomic sense RNAに相補
＊　性を有する配列

20

※配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域
の１２２～１４１番目の antigenomic sense RNAに相補
※　性を有する配列

TTTGAGGCTC TGTTTGCCTG

【００７６】配列番号：３４
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
GGACATATCC TGGCGCCCCG

【００７７】配列番号：３５
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
GGCGCTGCCA TTTCAGTGAA

【００７８】配列番号：３６
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
GGTGGAGCCA TCTCATGTAT

【００７９】配列番号：３７
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ
配列
CATTTCAGTG AAAATRTCGT

【００８０】配列番号：３８
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
CATCTCRTGT ATAACRTCCA

【００８１】配列番号：３９
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列
ATATCTTGAC GCCCAGCGCT

【００８２】配列番号：４０
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ

20

＊配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域の１２２～１４１番目の antigenomic sense RNAに相補
＊　性を有する配列

20

※配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域の１８１～２００番目の antigenomic sense RNAに相補
※　性を有する配列

20

★配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子の１８１～２００番目の antigenomic sense RNAに相補性を
★　有する配列

20

☆他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域の１７３～１９２番目の antigenomic sense RNAに相補性を有する配列。配列中、塩基番号１９番目のQは、Ｃ又はＴを示す。
☆

20

◆配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４蛋白をコードする遺伝子領域の１７３～１９２番目の antigenomic sense RNAに相補
◆　性を有する配列

20

＊配列の種類：他の核酸　合成ＤＮＡ
他の情報
エンテロウイルスのＶｐ４をコードする遺伝子領域の１１８～１３７番目の antigenomic sense RNAの相補性を
＊　有する配列

20

他の情報
エンテロウイルスのＶｐ４をコードする遺伝子領域の１７２～１９１番目の antigenomic sense RNAの相補性を有する配列。配列中、塩基番号３番目のQは、Ｃ又はＴを示す。

配列
ATGTCATGTA TAACRTCCAT

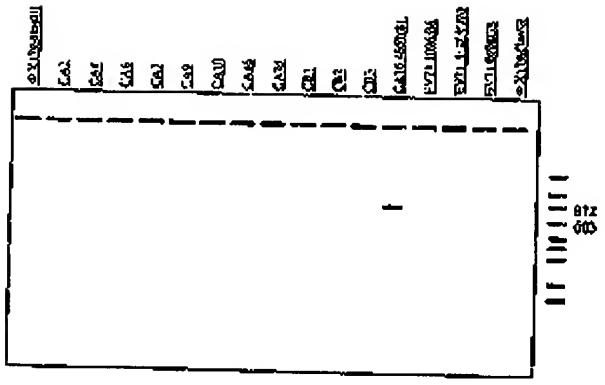
【００８３】配列番号：４１
配列の長さ：２０
配列の型：核酸
鎖の数：一本鎖
トポロジー：直鎖状
配列の種類：他の核酸　合成ＤＮＡ　＊
配列
ATTTCAGTGA AAATRTCGTT

【図面の簡単な説明】
【図１】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図２】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図３】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図４】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図５】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図６】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図７】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図８】ＣＡ１６特異プローブ（配列番号３２．配列番号３８及び配列番号３９）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図９】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。

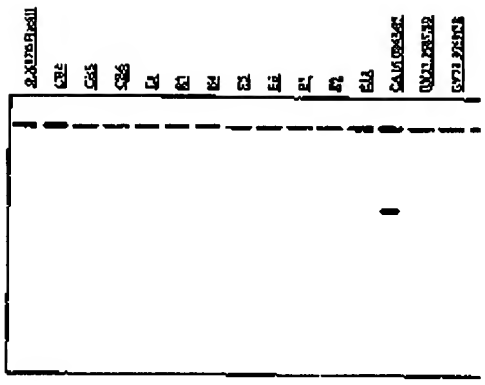
＊他の情報
エンテロウイルスのＶｐ４をコードする遺伝子領域の１７２～１９１番目の antigenomic sense RNAの相補性を有する配列。配列中、塩基番号１８番目のQは、Ｃ又はＴを示す。

【図１０】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１１】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１２】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１３】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１４】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１５】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１６】ＥＶ７１特異ＤＮＡプローブ（配列番号３１及び配列番号４１）と増幅ＤＮＡとのハイブリッド形成を示す図である。
【図１７】エンテロウイルスの増幅遺伝子領域の位置、増幅ＤＮＡの大きさ並びにＣＡ１６特異ＤＮＡプローブ及びＥＶ７１特異ＤＮＡプローブの結合位置を示す図である。図中２ケタの数字は配列番号．（　）内の数字はＶｐ４蛋白をコードする遺伝子領域内の位置、〔　〕内の数字はＤＮＡ断片の大きさ（ｂｐ）を示す。

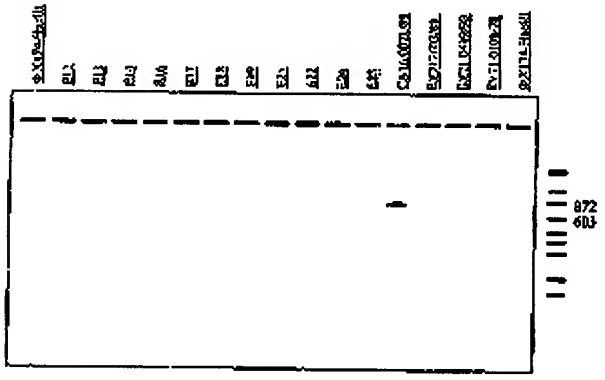
【図1】



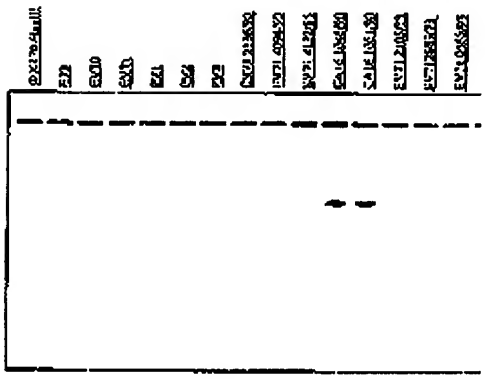
【図2】



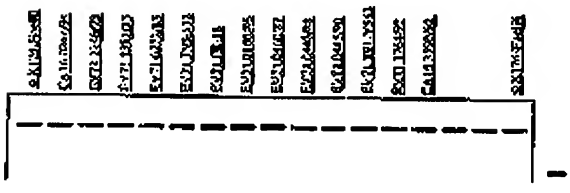
【図3】



【図4】

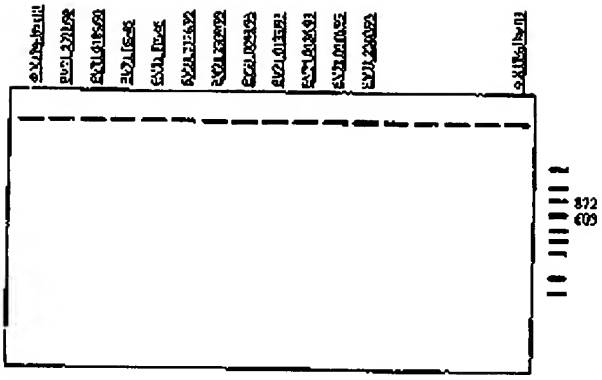


【図5】

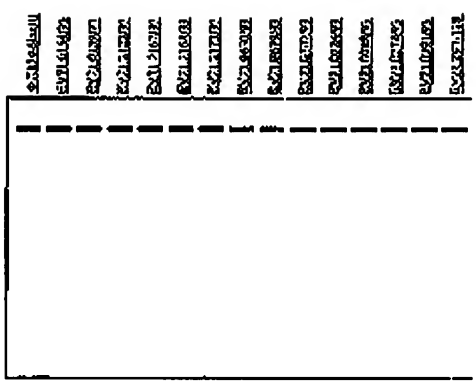


【図6】

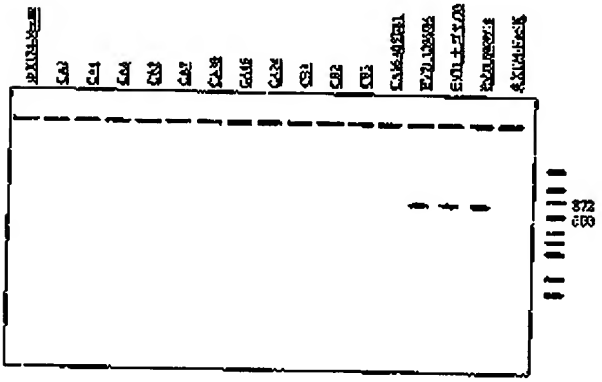
【図7】



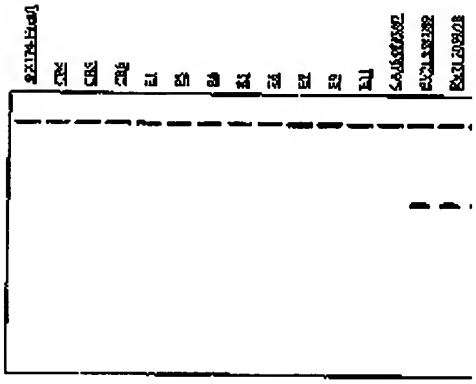
【図8】



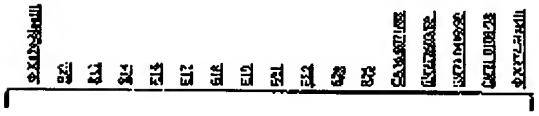
【図9】



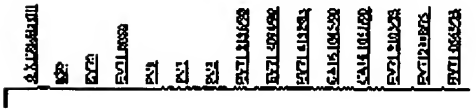
【図10】



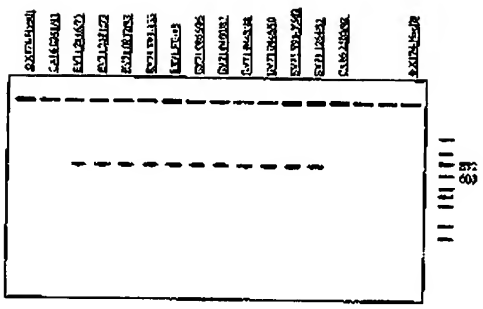
【図11】



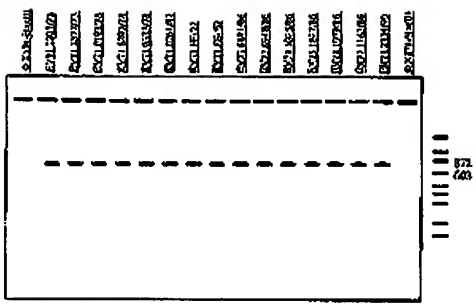
【図12】



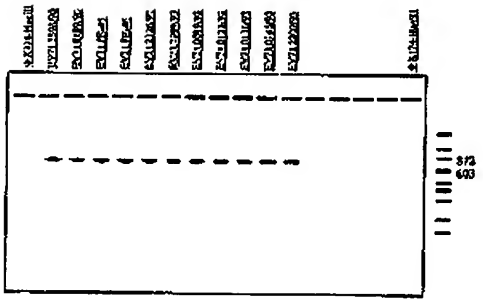
【図13】



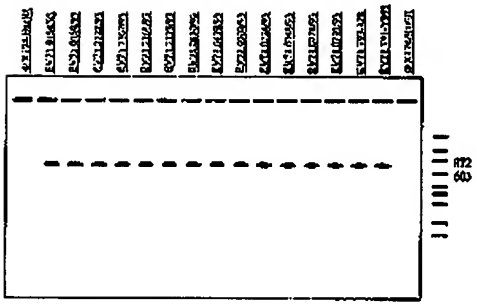
【図14】



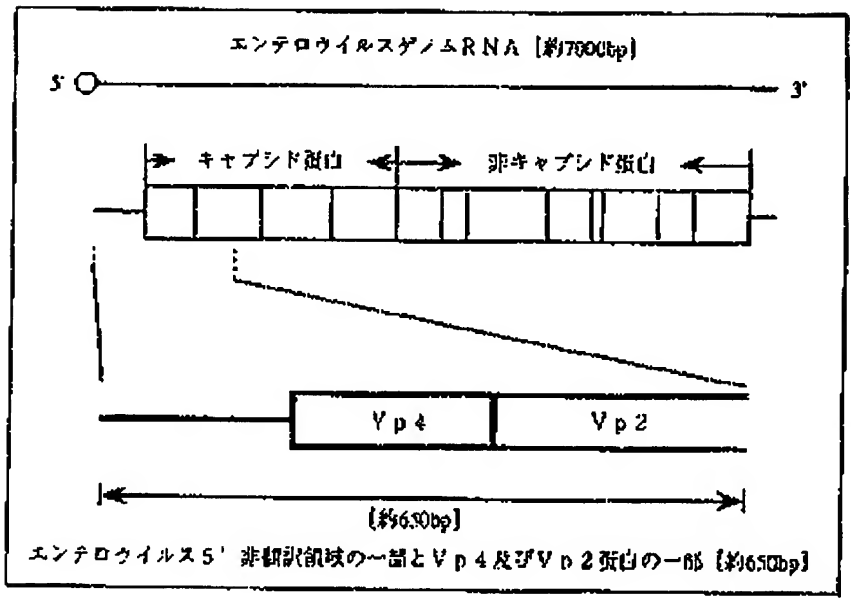
【図15】



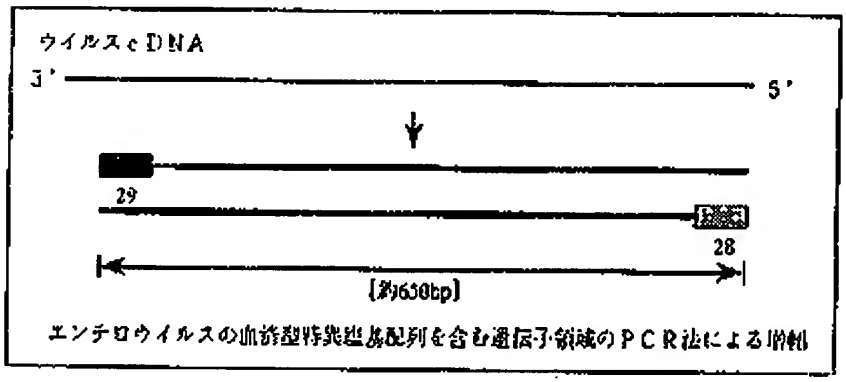
【図16】



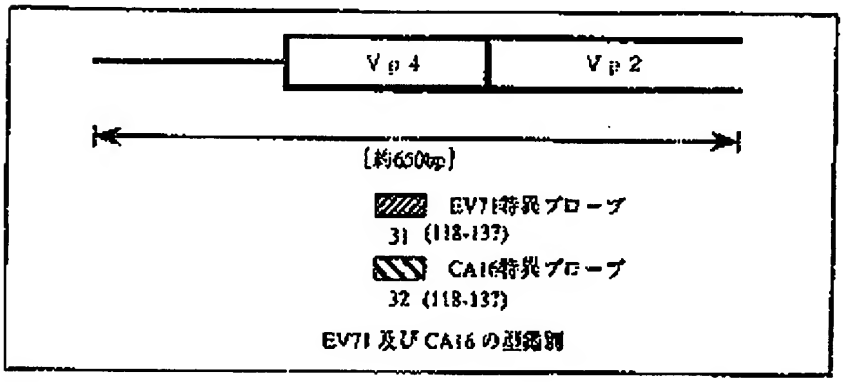
【図１７】



エンテロウイルス５' 非翻訳領域の一部とＶｐ４及びＶｐ２蛋白の一部【約650bp】



エンテロウイルスの血清型特異塩基配列を含む遺伝子領域のＰＣＲ法による増幅



EV71 及び CA16 の型鑑別